THE CHEMISTRY OF XANTHORRHOEA RESINS

by

COLIN JAMES DAHL

A thesis submitted in part fulfilment
of the requirements for the degree of

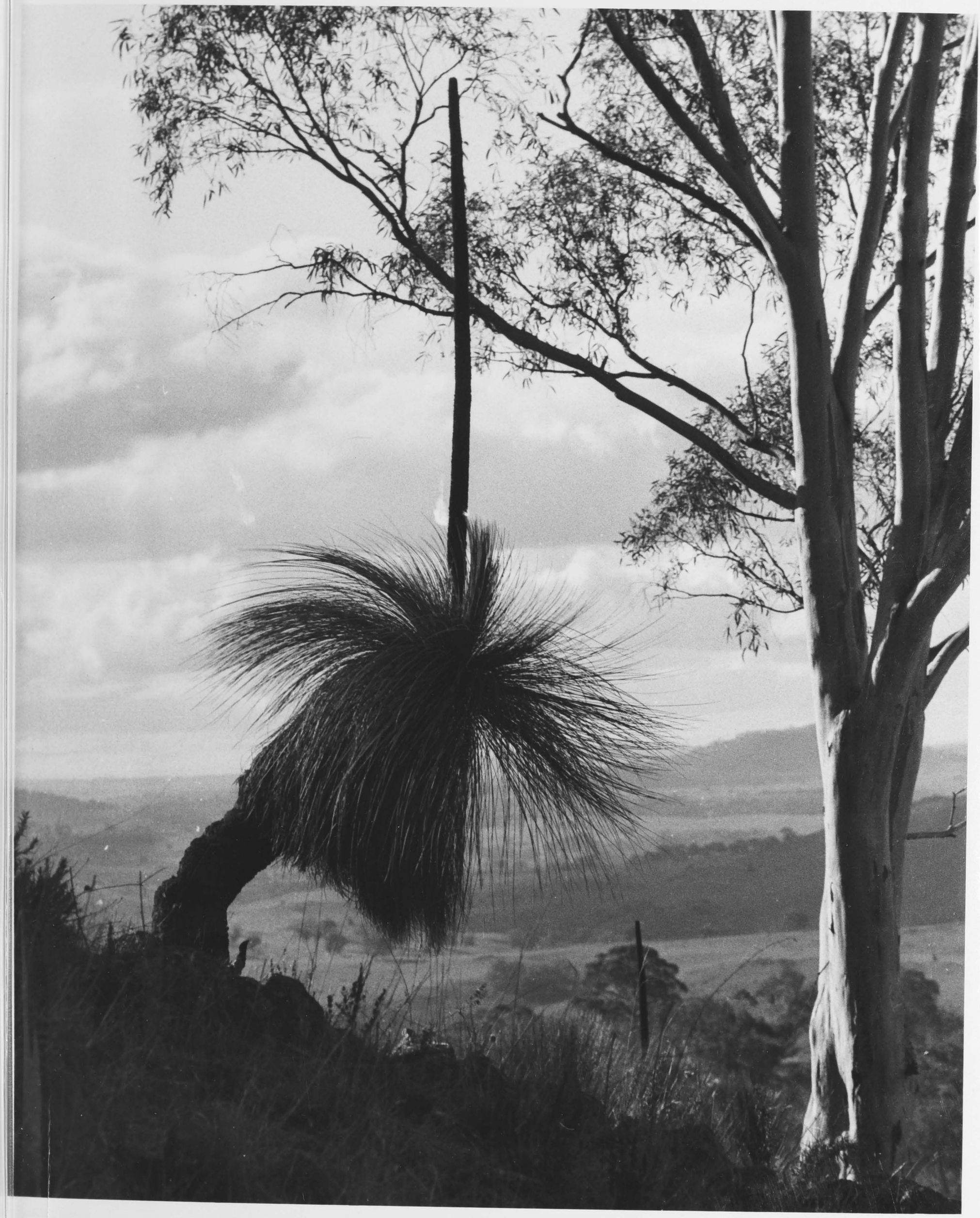
Doctor of Philosophy

in

The Australian National University

Research School of Chemistry,
The Australian National University,
Canberra, A.C.T.

April, 1969.



TO VERONICA

CONTENTS

Photograph : Specimen of Xanthorrhoea australis
(Mt. McDonald, A.C.T.)

PREFACE

The work described in this thesis is that of the candidate, except where otherwise stated, and has not been submitted for any other degree. It was carried out in the Department of Chemistry of The University of Manchester, England, and in the Research School of Chemistry of The Australian National University, Canberra, from 1966 to 1968.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisor, Professor A. J. Birch, for his guidance, perseverance and continual encouragement throughout this project; to Dr A. Pelter of The University of Manchester for his interest and assistance in the early part of this work and to my colleagues for helpful discussions.

I would also like to thank the spectroscopists, particularly Dr J. K. MacLeod and Dr R. Bramley, and the microanalysts and technicians in the Chemistry Departments of The University of Manchester and The Australian National University for their assistance.

Thanks are also due to The Australian National University for providing a scholarship.

SUMMARY

Samples of resin from X. preissii, X. australis and X. hastile have been examined. Two new naphthopyrans (5-hydroxy-2-methyl-2H,3H-naphtho[1,8-b,c]pyran and 3,5-dihydroxy-2-methyl-2H,3H-naphtho[1,8-b,c]pyran, and two new bis-flavonoids(xanthorrhone and hydroxyxanthorrhone) have been isolated and characterised. A number of compounds not previously found in the resins (pinocembrin, sakuranetin, isosakuranetin, hesperitin, aloe-emodin, 4',7-dimethoxy-5-hydroxyflavanone, 4,4'-dimethoxy-2',3,5-trihydroxychalcone and 2-phenylethyl phenylacetate) and other known compounds have also been identified. A synthesis of xanthorrhone by a method possibly related to the natural process has been carried out, and the biosynthetic and chemotaxonomic aspects of the investigation discussed.

INTRODUCTION

Grass trees, sometimes called "blackboys", especially in Western Australia, or yaccas, or yackas, are widely distributed throughout the Commonwealth of Australia. Characterised by their bunches of grass-like leaves growing at the top of a thick woody stem, like a palm, these trees belong to the family Xanthorrhoeaceae. The family, 8 genera and about 60 species, is endemic to Australia except for a few species of Lomandra found in New Caledonia and New Guinea. The genus Xanthorrhoea can be easily distinguished from the other genera which are usually much smaller and morphologically quite different.

Xanthorrhoeas are very slow growing and the soil in which they are found usually has a low nitrogenous plant food content. These perennials range in size from species so condensed as to barely appear above ground level (aculine) to more or less arborescent varieties seven metres high. The stem consists of a fibrous somewhat spongy material surrounded by a hard husk consisting of the bases of old leaves cemented together

with exuded resin. This resin, which is mainly red or yellow according to the species, is the subject of the present investigation.

Interest in this particular resin, in recent times anyway, arises from the fact that it is a potential source of biogenetically interesting compounds. Largely phenolic in nature the resin has been shown to contain a variety of compounds derived biosynthetically from various combinations of acetate and cinnamate units. As the number of compounds isolated from the resin has increased interesting structural patterns have emerged. This investigation was undertaken in the belief that a systematic examination of the resin using modern chromatographic and spectroscopic techniques would lead to a precise definition of these patterns and possibly a correlation of them with the various species. This would permit further insight to be gained into the biochemical mechanisms by which the composition of the resin is determined as well as providing a method for distinguishing between the species on purely chemical grounds.

REVIEW OF EARLIER WORK

Xanthorrhoea grass-trees have been the subject of numerous investigations since Lichenstein[1] first reported work on the topic in 1799. From that date up until about 1890 there were many attempts made to utilise various parts of the plant,[2] however, these investigations were carried out without any regard to the source or species under examination and from a chemical point of view the accounts are confusing and provide little useful information apart from some observations relating to solubility.

The first objective chemical investigations came in the 1890's when Bamberger[3] demonstrated the presence of cinnamic and p-coumaric acids and Tschirch and Hildebrand[4] noted the polyphenolic nature of the resins and isolated, in addition to these two acids, vanillin, styracin, phenylpropyl-p-coumarate and p-hydroxybenzaldehyde.

From 1900 to 1930 interest in Xanthorrhoea plants was quite widespread and many publications on the subject appeared. Many of the investigations were oriented exclusively to finding a commercially exploitable Xanthorrhoea product and the formidable list of possible

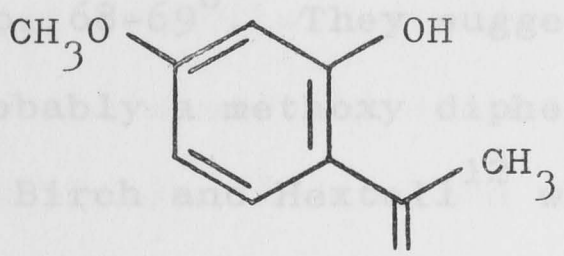
outlets which appeared[5] was remarkable not only in its length but in its diversity. Xanthorrhoeas were to figure in the manufacture of paper, perfumes, varnishes, soaps, munitions and even chewing gum. For a variety of reasons, however, none of these proposals proved economically feasible.

The purely chemical investigations of this period though perhaps not so numerous were nevertheless quite significant. In 1920 the Imperial Institute undertook an examination of samples of red and yellow resin. Extraction and purification of these yielded small quantities of cinnamic and coumaric acids along with complex phenolic material, while both resins on oxidation with nitric acid produced good yields (25-30 per cent) of picric acid.

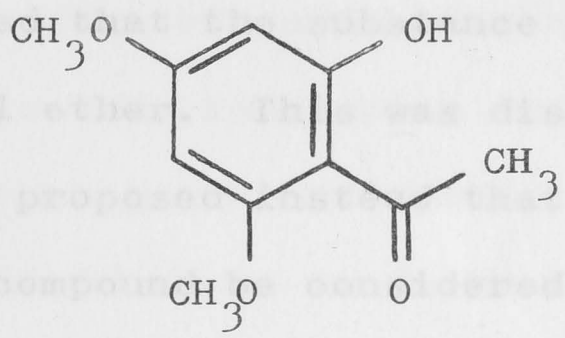
Attention was also drawn to other portions of the plant. Mann[7] obtained yields of alcohol up to 1.24 per cent (of the trunk) by fermenting the aqueous extract of the core with yeast. He claimed that the core of X. preissii contained 50 per cent carbohydrates including 10 per cent reducing sugars and 16 per cent non-reducing sugars. Rowley[8] and Lightfoot[9] put forward other figures, but unfortunately although this work was completed more than 40 years ago the matter has not been

investigated further, and no attempt has been made to determine the detailed composition of the carbohydrate extractives.

The most important work of the time was that carried out in the 1920's by Rennie, Cooke and Finlayson.[10,11] They examined the steam volatile material from alkaline solutions of a red resin (Kangaroo Is.), X. tateana (Kangaroo Is.), X. preissii, X. arborea, X. hastile* and X. reflexa and found that with the exception of X. hastile, which gave citronellol (8 per cent) and cinnamyl alcohol (53 per cent), all the resins were found to produce paeonol (I) and "hydroxypaeonol". The latter originally formulated as



I

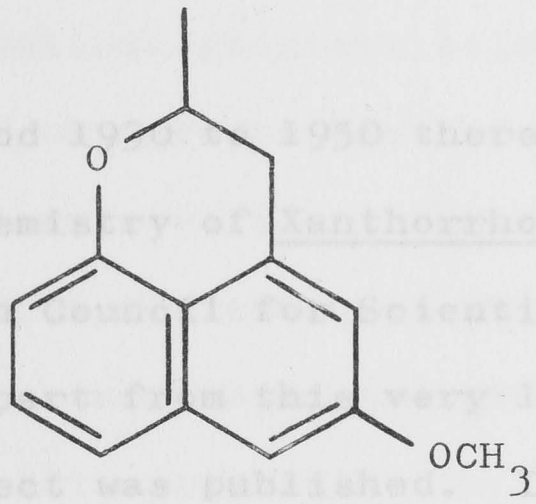


II

---

* According to the botanists "X. hastile" is synonomous with X. resinosa, ssp. resinosa. The name appears to have been misapplied to many other species, however, so the term will be taken to mean "yellow resin" as distinct from the darker red or brown resin of most other species. The matter will be fully discussed later.

$C_9H_{10}O_4$, was subsequently shown by Birch and Hextall[12] to be 2-hydroxy-4, 6-dimethoxy acetophenone (II).



III

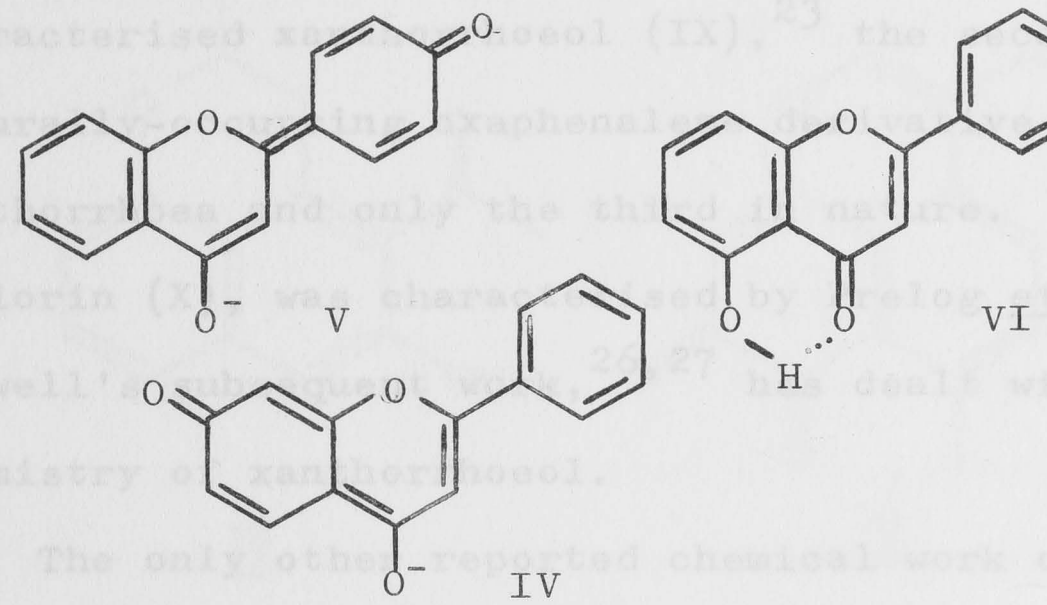
In addition, Finlayson et al.[10,11] found that the distillate from two of the resins, X. reflexa and X. preissii contained a colourless crystalline compound m.p. 68-69°. They suggested that the substance was probably a methoxy diphenyl ether. This was discounted by Birch and Hextall[12] who proposed instead that on the basis of u.v. spectra the compound be considered as a methoxymethylnaphthodihydrofuran. This also proved to be incorrect and it was in fact some 40 years after its initial isolation that the full structural details (apart from stereochemistry) of the compound, xanthorrhoein, were disclosed by the degradative and synthetic work of Birch, Smith and Salahud-Din.[14] They

showed that xanthorrhoein was in fact a methoxy methyl derivative of dihydro-oxaphenalene(III) and as such the first naturally-occurring oxaphenalene derivative to be isolated.

In the period 1930 to 1950 there was a useful review of the chemistry of Xanthorrhoea resin compiled by the Australian Council for Scientific and Industrial Research[15] but apart from this very little significant work on the subject was published. In the 1950's, refined extraction procedures and chromatographic techniques were brought to bear on the problem. Ryan,[16] Hextall[17] and McReavie,[18] presumably noting the earlier observation by Cooke[19] that the solubility of the resin greatly increased with increasing pH, found that the resin could be conveniently divided into bicarbonate, carbonate and alkali-soluble fractions which on methylation and column chromatography produced crystalline materials. Some of these on further purification were identified as flavonoids.

This method of separation, which now forms the basis for present day investigations, owes its success to the fact that flavonoid derivatives exhibit degrees of acidity depending on their substitution pattern. Briggs and Locker[20] have shown that phenoxide ions

produced at the 7 or 4' positions of flavone are resonance stabilised by the carbonyl group as in structures (IV) and (V), and flavones hydroxylated in these positions exhibit greatest acidity. In fact, when both 7 and 4' hydroxy groups are present the flavone may be soluble in a saturated bicarbonate solution. On the other hand, 5-hydroxyflavones, as in(VI), have a strongly hydrogen bonded hydroxyl group which exhibits only very weak acidic properties.

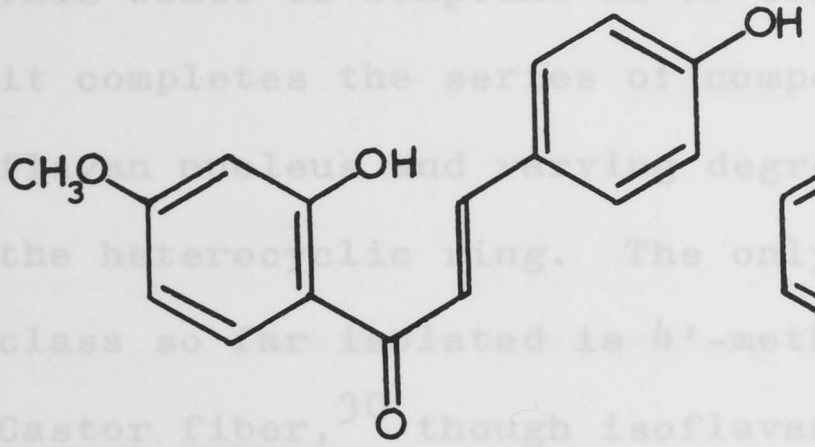


From his studies on paper chromatography Simpson[21] deduced that the acidities of flavone hydroxy groups decrease in the order 7>4'>3'>3 and it is therefore quite apparent that a mixture of flavones with various substitution patterns can be fairly effectively separated into groups on this basis. These principles

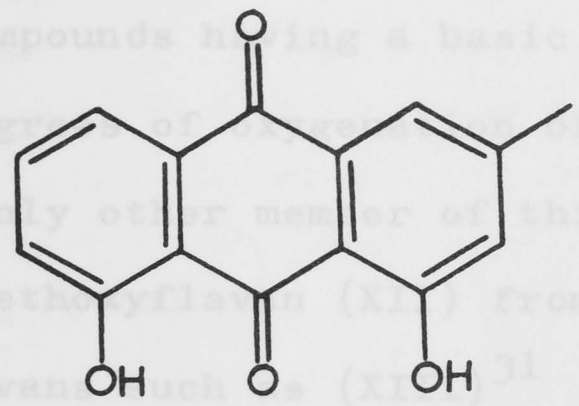
apply to other benzopyrone systems, but in the case of isoflavones and dihydroflavones only A-ring hydroxylation can be involved in resonance stabilisation.

In 1954 an independent investigation into Xanthorrhoea resin was initiated by Duewell. This resulted in the isolation of a 6'-deoxychalcone (VII) along with chrysophanic acid (VIII); the latter considered to be an artefact occurring in bushfire damaged material.[22] Later he isolated and fully characterised xanthorrhoeol (IX),[23] the second naturally-occurring oxaphenalene derivative from a xanthorrhoea and only the third in nature. The other, biflorin (X), was characterised by Prelog et al.[24,25] Duewell's subsequent work,[26,27] has dealt with the chemistry of xanthorrhoeol.

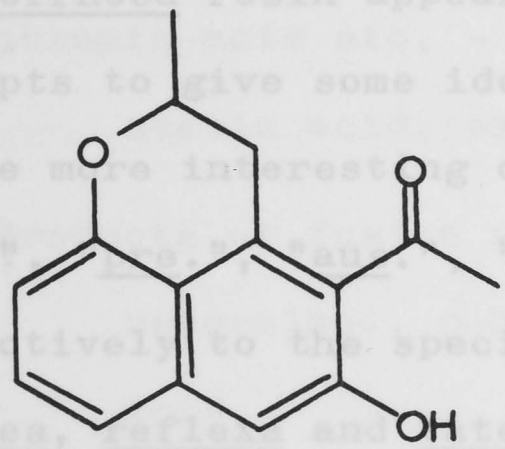
The only other reported chemical work on Xanthorrhoea resins to the present time has been carried out by Salahud-Din.[28] In addition to his synthetic work with Birch on xanthorrhoein, previously mentioned, he examined a sample of resin thought to be X. preissii and succeeded in isolating a number of flavonoids from the methylated caustic-soluble fraction. One of these was subsequently identified as 4', 5, 7-trimethoxyflavan (XI), the first naturally-occurring flavan to be isolated.



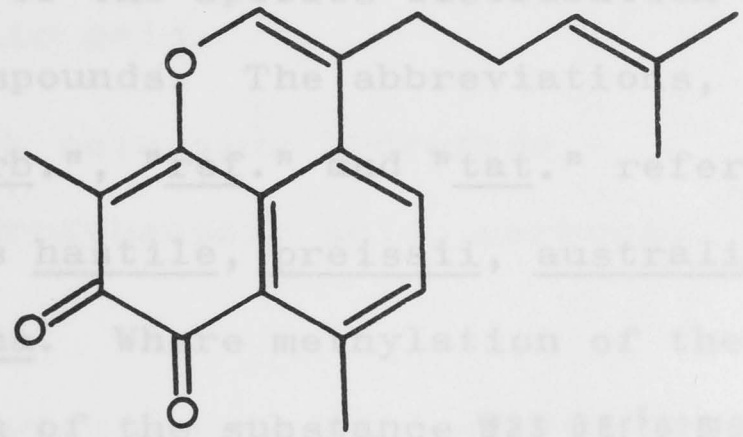
VII



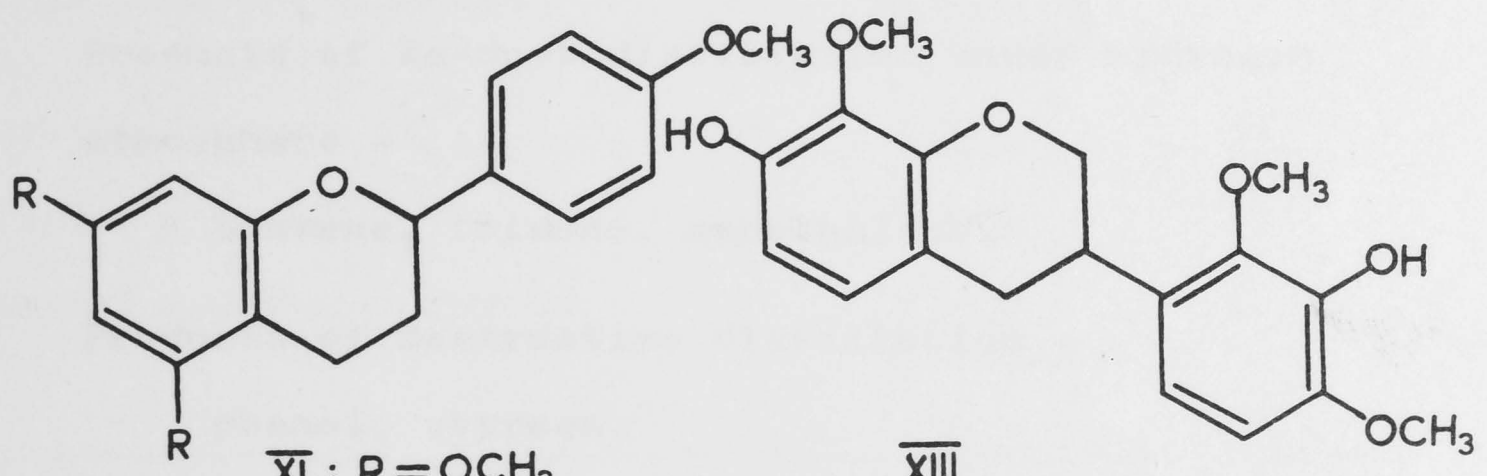
VIII



IX



X



XI : R = OCH_3

XII : R = H

XIII

This class of compound is of considerable interest as it completes the series of compounds having a basic flavan nucleus and varying degrees of oxygenation of the heterocyclic ring. The only other member of this class so far isolated is 4'-methoxyflavan (XII) from Castor fiber,[30] though isoflavans such as (XIII)[31] are known.

A comprehensive list of substances obtained from Xanthorrhoea resin appears in Tables 1 and 2. Table 2 attempts to give some idea of the species distribution of the more interesting compounds. The abbreviations, "has.", "pre.", "aus.", "arb.", "ref." and "tat." refer respectively to the species hastile, preissii, australis, arborea, reflexa and tateana. Where methylation of the fraction prior to isolation of the substance was performed the word "methylated" appears in parenthesis.

TABLE 1

1. Acids -

benzoic, cinnamic, p-coumaric.

2. Aldehydes -

vanillin, p-hydroxybenzaldehyde.

3. Products of oxidation by alkaline permanganate, chromic acid etc. -

acetic acid, oxalic acid.

4. Products of fusion with potassium hydroxide -

resorcinol, p-hydroxybenzoic acid, carbonic acid.

5. Products of oxidation with nitric acid -

picric acid, p-nitrophenol, acetic acid.

6. Products of Zn-dust distillation under hydrogen atmosphere -

benzene, toluene, naphthalene.

7. Products of destructive distillation -

phenol, styrene.

TABLE 2

Compound	m.p.	Source	Reference
citronellol	(b.p.222)	X.has.	11
		X.pre.	10
cinnamic acid	133	X.has.	18,5(c)
		X.aus.	5(c)
methyl cinnamate	37	X.has.	18
cinnamyl alcohol	33	X.has.	18,11
p-coumaric acid	210	X.has.	18
		X.ref.	16
		X.tat.	10
		X.pre.	10
methyl p-coumarate	136	X.has.	18
p-methoxy-cinnamic acid	171	X.has. (methylated)	18
methyl p-methoxy-cinnamate	89	X.has. (methylated)	18
cinnamyl cinnamate (?)*	88	X.has.	18
cinnamyl p-coumarate	106	X.has.	18
cinnamyl p-methoxy-cinnamate	169	X.has. (methylated)	18

* synthetic material has m.p. 44°.

Contd.

Compound	m.p.	Source	Reference
2-hydroxy-4-methoxyacetophenone [paeonol]	52	X.tat.	5(c),10
		X.pre.	5(c),10
		X.arb.	11
		X.ref.	11
4,6-dimethoxy-2-hydroxyaceto-phenone	82	X.tat.	5(c),10
		X.pre.	5(c),10
		X.arb.	11,12
		X.ref.	11,12
2',4-dihydroxy-4'-methoxychalcone	175	X.aus.	22
		X.ref.	16
4',6'-dimethoxy-2'-hydroxychalcone	89	X.pre. (methylated)	28
2'-hydroxy-4,4',6'-trimethoxychal-cone	113	X.pre. (methylated)	16,17,28
2'-hydroxy-3,4,4'-trimethoxychal-cone	158	X.ref. (methylated)	16
5,7-dimethoxyflavanone*	170	X.pre. (methylated)	28
4',7-dihydroxyflavanone	207	X.arb.	32
7-hydroxy-4'-methoxyflavanone	184	X.ref.	17
5,7,4'-trihydroxyflavanone	251	X.pre.	17,28
		X.arb.	32
4',7-dihydroxy-5-methoxyflavanone	263	X.pre.	17,28
4',7-dimethoxy-5-hydroxyflavanone	117	X.pre. (methylated)	17
4',5,7-trimethoxyflavan	109	X.pre. (methylated)	28

* synthetic material has m.p. 143-144°.

Contd.

Compound	m.p.	Source	Reference
chrysophanic acid	196	X.aus.	22
2-hydroxy-3',4,4',6-tetramethoxy-benzophenone	178	X.has. (methylated)	16,18
xanthorrhoein	68	X.ref.	11,16
		X.pre.	10,12,14
xanthorrhoeol	121	X.arb.	23,26,27
		X.pre.	28

RESULTS AND DISCUSSION

PART I

GENERAL APPROACH

In this investigation samples of resin from X. preissii, X. australis and X. hastile have been examined. The methods adopted for extraction and initial fractionation of the resin have been based on those of previous workers (i.e. extraction with acetone followed by removal of solvent, addition of ether and fractionation on the basis of acidity), but there have been some significant departures from earlier procedures. Table 2 has provided a general indication of the types of compound present in the resin but it does draw attention to two problems. Firstly, we cannot be sure that the isolation of compounds of a particular class such as chalcones, by the methods used previously, genuinely indicates their presence in the original resin as these can arise from base treatment of flavanones. Secondly, there is no way of discerning from the isolated methylated compounds the extent to which methylation had previously occurred in vivo.

It has been necessary in this investigation therefore to modify procedures in such a way that the possibility of artefacts arising would be minimised and the results obtained interpretable in the broadest possible manner. To that end the following steps have been taken:- (a) Where possible the resin was selected from that part of the trunk which was considered least likely to have been affected by bushfires. (b) All solvents were removed under reduced pressure at a temperature below 50^{o}. (c) The extracts were initially divided up into bicarbonate-soluble, carbonate-soluble and carbonate-insoluble fractions. Extraction with alkali was not carried out until the carbonate-insoluble fraction had been examined for the presence of chalcones. When it was carried out, ice-cold solutions of 5 per cent sodium hydroxide were used and these were neutralised as soon as possible after the extraction. (d) Solid carbon dioxide was used to neutralise solutions where practicable thus minimising the use of mineral acid which could affect compounds such as flavanols. (e) In general, fractions were not methylated before chromatography.

This approach made the problem of separating the "intractable resin" into pure components somewhat more

difficult. Heavy reliance was placed on the development of suitable gas, column and thin-layer chromatographic systems and techniques, and it is perhaps significant that in many ways the problems associated with these and other purification methods often assumed greater proportions than the frequently straightforward problem of structure elucidation. Nevertheless, the methods used yielded some useful results and a number of new and interesting compounds were isolated.

PART II

EXAMINATION OF THE RESIN FROM XANTHORRHOEA PREISSII

A sample of plant material thought to be X. preissii was obtained from the Manchester Museum, England. This material was broken apart and extracted with a large volume of cold acetone. The extract was worked up in the usual way* and the resulting ethereal solution concentrated to a convenient volume and then successively extracted with aqueous solutions of potassium bicarbonate (10 per cent) and sodium carbonate (10 per cent). A small sample of the carbonate-insoluble fraction was extracted with alkali to determine the extent of the neutral fraction. Results indicated that the resin could be divided up as follows:-

Bicarbonate-Soluble Fraction	1 per cent
Carbonate-Soluble Fraction	20 per cent
Alkali-Soluble Fraction	70 per cent
Neutral Fraction	3 per cent

The bicarbonate-soluble fraction consisted of a small amount of an extremely complex mixture. This was discarded.

---

* For details cf. Experimental, p.169.

The carbonate-soluble fraction, a viscous dark brown tar, was extracted with warm benzene. On cooling, the benzene solution was decanted and evaporated under reduced pressure to yield a brownish amorphous solid. A sample of this solid was taken up in benzene and chromatographed on a silicic acid column with benzene as the eluting solvent. 10 ml. fractions were collected, the solvent evaporated from each under reduced pressure, and the u.v. absorption spectrum (in ethanol) of the individual fractions recorded. Fraction PC18, the purest spectroscopically, was examined further.

PC18: 5,7-Dihydroxyflavanone. [Pinocembrin]

Obtained as a pale yellow semicrystalline solid the material from this fraction could be readily recrystallised from aqueous alcohol. After several recrystallisations from this medium it was obtained as colourless needles m.p. 199-200°.

The material was examined by thin-layer chromatography on silica gel and shown to be free from contamination. It could be detected on a t.l.c. plate by spraying with concentrated sulphuric acid then applying gentle heat until it appeared as a deep yellow spot. The compound exhibited u.v. absorption spectra normally characteristic of flavanones. $\lambda_{max.}$ (Ethanol)

occurred at 290 mμ with a shoulder at 325 mμ. The addition of alkali* produced a bathochromic and hyperchromic shift[33] of 38 mμ to 328 mμ, while the addition of aluminium chloride caused a shift[34] in the $\lambda_{max.}$ to 310 mμ, thereby indicating the formation of an aluminium complex. Sodium acetate was found to be sufficiently basic to produce a stable anionic form,[35] evidenced by the clear resolution of the shoulder at 325 mμ into a peak at 328 mμ, so it was deduced that the compound could form a phenoxide ion capable of resonance stabilisation through a carbonyl group. I.r. absorption spectra indicated a strongly hydrogen-bonded carbonyl group, the band appearing at 1630 cm^{-1}.

On the basis of the above evidence it was concluded that the substance was a flavanone possessing at least two hydroxy groups; one at the 5-position, the other at the 7-position. The compound did not, however, give a positive Shinoda test[36] (Mg/HCl) nor did it produce red colouration on treatment with borohydride followed by acid. Examination of a number of flavanones revealed that compounds which do not possess an oxygen substituent in the B-ring, such as flavanone itself and 5,7-dimethoxyflavanone give negative tests under these conditions.

*i.e. 2N NaOH

The absence of any other substituents on the flavanone nucleus was clearly shown by the 60 Mc./sec. p.m.r. spectrum in deutero-acetone. Seven aromatic proton absorptions were observed, the five B-ring protons as a multiplet at τ2.5, and C-6 and C-8* protons of the A-ring together as a singlet at τ3.95. Aliphatic proton absorptions at τ 7.0 (multiplet) (2H) and τ 4.40 (quartet) (1H)($J_{2,3a}$ = 11.5 c/s; $J_{2,3e}$ = 4.5 c/s; $J_{3e,3a}$ = 16.5 c/s) corresponded, respectively, to those on C-3 and C-2. The phenolic protons produced a sharp one-proton peak at -τ2.26 (hydrogen-bonded) and a one-proton peak at τ6.16.

The mass spectra of flavanones have been well documented[37] and the material under investigation produced the usually observed fragmentation pattern (Scheme 1). The major pathways involved breakdown by a reverse Diels-Alder process, with and without hydrogen transfer, and the loss of either a hydrogen atom or aryl radical from the molecular ion to give even electron fragments. Further losses of CO then occurred.

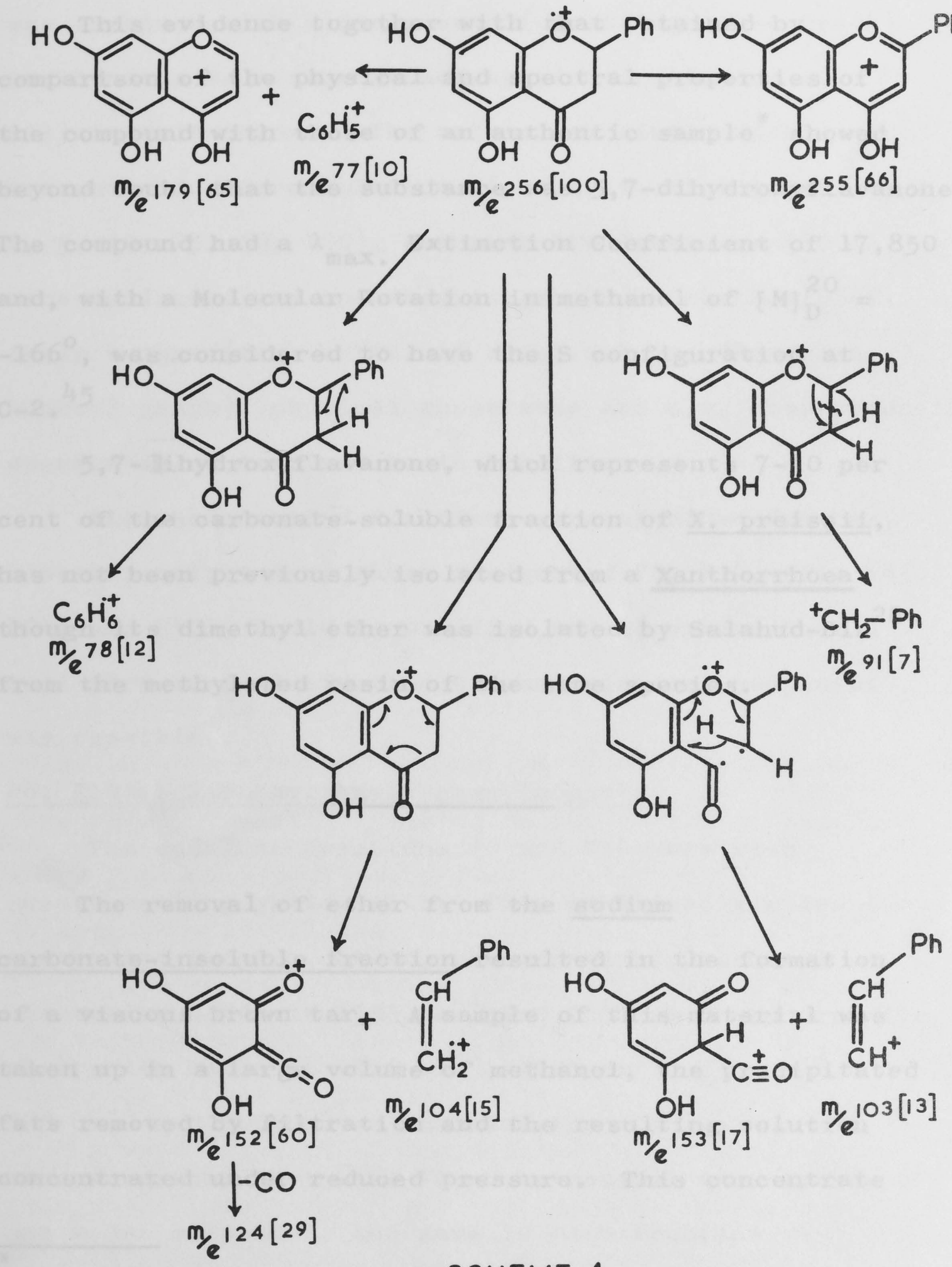
---

* Throughout this thesis the following notation will be used:

C-1 for carbon-1, etc.

H-1 for the proton on carbon-1, etc.

(1H) for an integration of one proton in the p.m.r. spectrum.


HO
O
+
OH OH
m/e 179 [65]
+
C6H5+
m/e 77 [10]
HO
O+
Ph
OH O
m/e 256 [100]
HO
O
Ph
+
OH OH
m/e 255 [66]
HO
O+
Ph
H
H
OH O
C6H6+
m/e 78 [12]
+CH2—Ph
m/e 91 [7]
HO
O+
Ph
H
H
OH O
HO
O+
C=O
OH
m/e 152 [60]
-CO
m/e 124 [29]
CH
CH2+
m/e 104 [15]
HO
O
H
C≡O+
OH
m/e 153 [17]
CH
CH+
m/e 103 [13]

SCHEME 1

This evidence together with that obtained by comparison of the physical and spectral properties of the compound with those of an authentic sample* showed beyond doubt that the substance was 5,7-dihydroxyflavanone. The compound had a $\lambda_{max.}$ Extinction Coefficient of 17,850 and, with a Molecular Rotation in methanol of $[M]_D^{20}$ = -166^o, was considered to have the S configuration at C-2.[45]

5,7-Dihydroxyflavanone, which represents 7-10 per cent of the carbonate-soluble fraction of X. preissii, has not been previously isolated from a Xanthorrhoea though its dimethyl ether was isolated by Salahud-Din[28] from the methylated resin of the same species.

The removal of ether from the sodium carbonate-insoluble fraction resulted in the formation of a viscous brown tar. A sample of this material was taken up in a large volume of methanol, the precipitated fats removed by filtration and the resulting solution concentrated under reduced pressure. This concentrate

---

* Kindly supplied by Professor H. Erdtman.

was combined with silicic acid (chromatographic grade) and benzene as a slurry, and applied to the top of a silicic acid column. The column was eluted with benzene-chloroform solutions (per cent chloroform: 0, 1, 2, 5, 10, 25, 50, 100), and over 400 fractions (20 ml.) were collected and combined in groups on the basis of their weight (from a "Wt. of fraction" vs. "Fraction number" graph), physical appearance and u.v. absorption spectra in neutral and basic conditions.

All the combined fractions were examined further, however, only a few, those designated F8, F14, F22, F45, F245 and F401, could be purified to the extent that a full structure elucidation of the principal component was possible.

F8, F14 : 2-Phenylethyl phenylacetate

The combined fractions F8 and F14 were both sweet-smelling yellow oils. They exhibited similar i.r. absorption spectra, having characteristic carbonyl absorption bands at 1735 cm^{-1} and 1250 cm^{-1} and aromatic absorption bands at 1600 cm^{-1}, 1495 cm^{-1}, 750 cm^{-1}, 725 cm^{-1} and 700 cm^{-1}. When examined by gas chromatography they were found to consist essentially of one major component, the same in both fractions. Purification of F14 was achieved by distillation under

reduced pressure (0.3 mm) from an oil bath, when a virtually colourless oil b.p. 155-160° was obtained.

The u.v. spectrum of this oil showed only benzenoid absorption about 258 mμ and its i.r. absorption spectrum suggested strongly that it was an aromatic ester. A 100 Mc./sec. p.m.r. spectrum (neat liquid) showed two 1:2:1 triplets at τ5.89 (2H)(J = 7.0c/s) and τ7.36 (2H)(J = 7.0 c/s), a sharp singlet at τ6.64 (2H) and a multiplet of 10 aromatic protons at τ2.9.

The evidence to this point suggested that the following structural units were present:

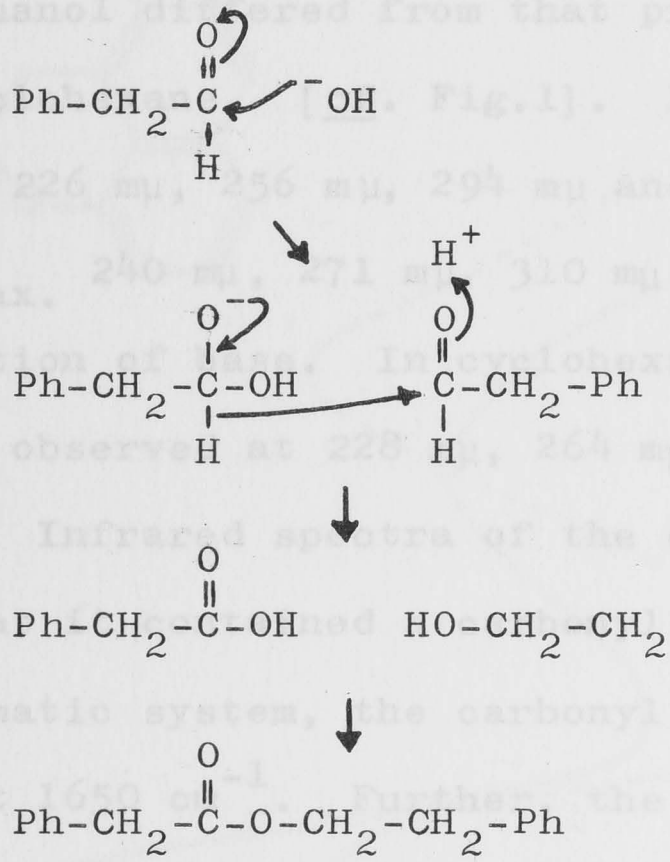
$$2 \times C_6H_5- \; ; \; -CH_2-CH_2- \; ; \; -\overset{O}{\overset{\|}{C}}-O- \; ; \; -CH_2-$$

Hydrolysis of F14 was achieved by treating a solution of the oil in ethanol with 10% aqueous sodium carbonate. The acid fraction on recrystallisation from water yielded colourless plates, m.p. 73-75°, subsequently identified as phenylacetic acid. (lit.[68] 76°). The alcohol fraction contained material having the same retention time on a gas chromatogram as authentic 2-phenylethanol.

A mass spectrum of the original oil indicated a molecular weight of 240 and the compound was then formulated as 2-phenylethyl phenylacetate. This

structure was confirmed by the synthesis of authentic material from 2-phenylethanol and phenylacetic acid and comparison of its physical and spectral properties with those of purified F14.

2-Phenylethyl phenylacetate has not been previously isolated from a Xanthorrhoea resin. It could possibly have been formed by a "Cannizzaro-type" reaction from phenylacetaldehyde obtained by oxidation of a C_6-C_3 unit.



F45: 4-Acetyl-5-hydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran. [Xanthorrhoeol]

The combined fraction F45 was obtained as a yellow crystalline solid. This was purified by recrystallisation

from a hexane-light petroleum (b.p. 60-80°) solution to give yellow "warts", m.p. 102-103°, or by recrystallisation from aqueous alcohol to give colourless plates, m.p. 121°. The yellow and colourless materials were indistinguishable by t.l.c., both having an Rf value of 0.3 (Silica gel H; chloroform), exhibiting a strong yellow fluorescence in ultraviolet light, and coupling with bis-diazotized benzidine to give a purple dye.[23]

Interestingly, the u.v. spectrum produced by both forms in ethanol differed from that produced by both forms in cyclohexane. [cf. Fig.1]. In ethanol, $\lambda_{max.}$ occurred at 226 mμ, 256 mμ, 294 mμ and 346 mμ with a shift to $\lambda_{max.}$ 240 mμ, 271 mμ, 310 mμ (infl.) and 370mμ on the addition of base. In cyclohexane, absorption maxima were observed at 228 mμ, 264 mμ, 316 mμ, 329 mμ and 412 mμ. Infrared spectra of the compound (in CCl_4) revealed that it contained a carbonyl group conjugated with an aromatic system, the carbonyl absorption band occurring at 1650 cm^{-1}. Further, the position of this band remained independent of concentration so the carbonyl group was involved in intramolecular hydrogen bonding.[38]

A 60 Mc./sec. p.m.r. spectrum of purified F45 in deuterochloroform revealed that the compound contained

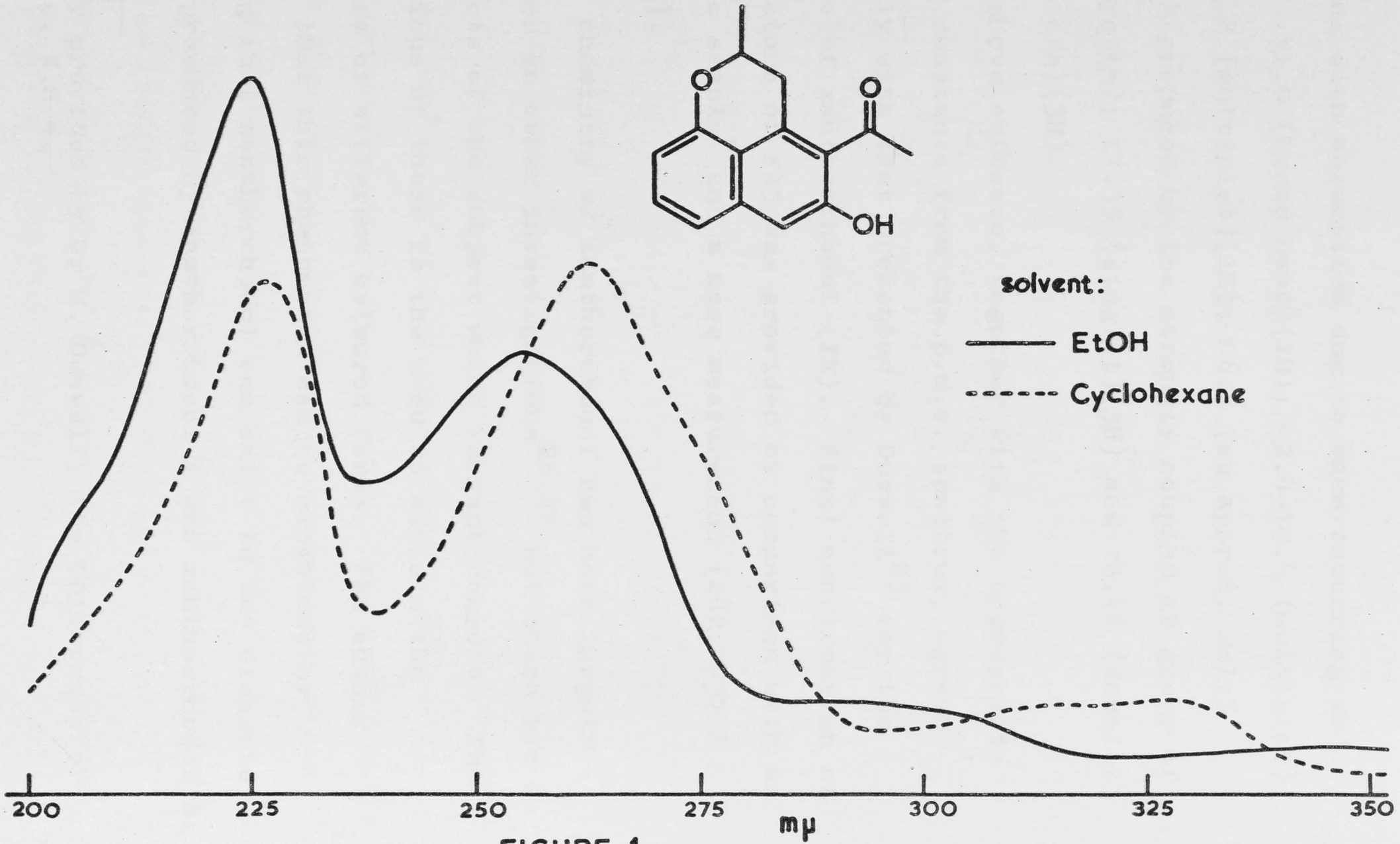


FIGURE 1

14 protons with absorption due to them occurring as follows: τ1.0 (broad band)(1H); τ 2.4-τ3.4 (multiplet) (4H); τ5.8 (multiplet)(1H); τ 6.8 (an approx. 2:1:1 "triplet" produced by the strongly coupled AB group of an ABX system); τ7.35 (singlet)(3H) and τ8.46 (doublet) (J = 6.0 c/s)(3H).

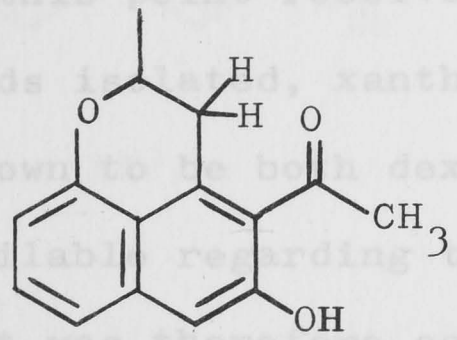
The above evidence, together with the appropriate coupling constants from the p.m.r. spectrum, agreed completely with that presented by Duewell[23] for the structure of xanthorrhoeol (IX). Final confirmation of the structure of F45 was provided by comparison with an authentic sample* and a mass measurement (242.0937 ≡ $C_{15}H_{14}O_3$).

The chemistry of xanthorrhoeol has been largely elucidated in other investigations[26,27] but there are a few aspects of the subject which warrant comment. The most obvious of these is the need to explain the occurrence of different coloured forms. The author suggests that this phenomenon can be explained by supposing that xanthorrhoeol can exist in two discrete forms represented by xanthorrhoeol A and xanthorrhoeol B.

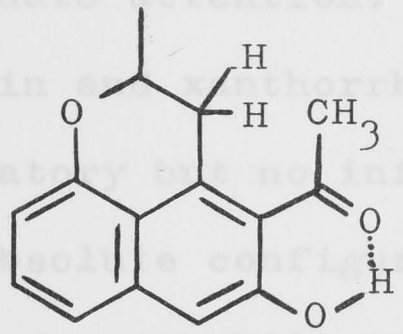
---

* Kindly provided by Dr H. Duewell, The University of Newcastle, N.S.W.

The factors considered most likely to determine which form will predominate are the steric interactions of acetyl methyl hydrogens with those on C-3, and the hydrogen bonding between the carbonyl group and the 5-hydroxy group.



Xanthorrhoeol A



Xanthorrhoeol B

When the compound is recrystallised from a polar solvent intermolecular bonding to the solvent molecules is far more important than intramolecualr hydrogen bonding, the acetyl group is free to adopt the sterically preferred form as in xanthorrhoeol A and the resulting product is colourless. Recrystallisation from a non-polar solvent, however, causes the carbonyl group to be directed towards the most favoured position for intramolecular bonding, despite some steric interaction, and this results in the formation of xanthorrhoeol B

having a suitable system for extensive electron delocalisation and hence highly coloured.

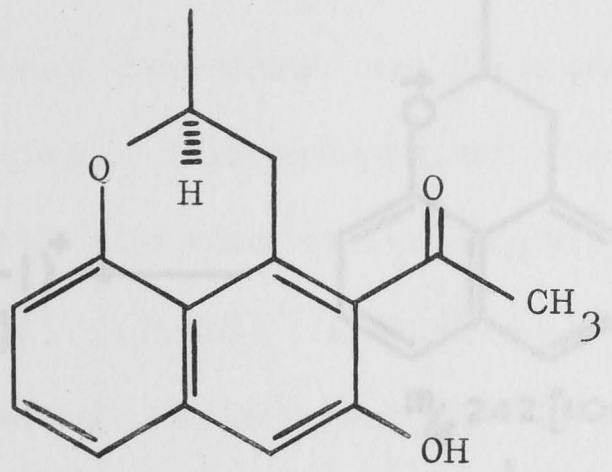
Similar considerations satisfactorily explain the observed u.v. and i.r. absorption phenomena.

The stereochemistry of compounds of this class had not to this point received adequate attention. The two compounds isolated, xanthorrhoein and xanthorrhoeol, were shown to be both dextrorotatory but no information was available regarding their absolute configuration at C-2. It was therefore considered worthwhile to undertake an investigation of this problem with the aim of degrading the molecule, without destroying the asymmetric centre, to a smaller molecule of know rotation and configuration.

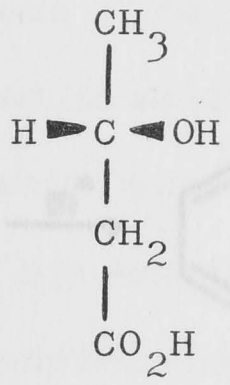
Exhaustive ozonolysis of xanthorrhoeol in methanol-chloroform solution followed by oxidative work-up produced a low yield of material indistinguishable on t.l.c. from an authentic sample* of β-hydroxybutyric acid (XIVa). (Silica gel H, fluorescent grade; water-saturated-ether/formic acid, 7:1; Rf = 0.7).

---

* racemic modification.



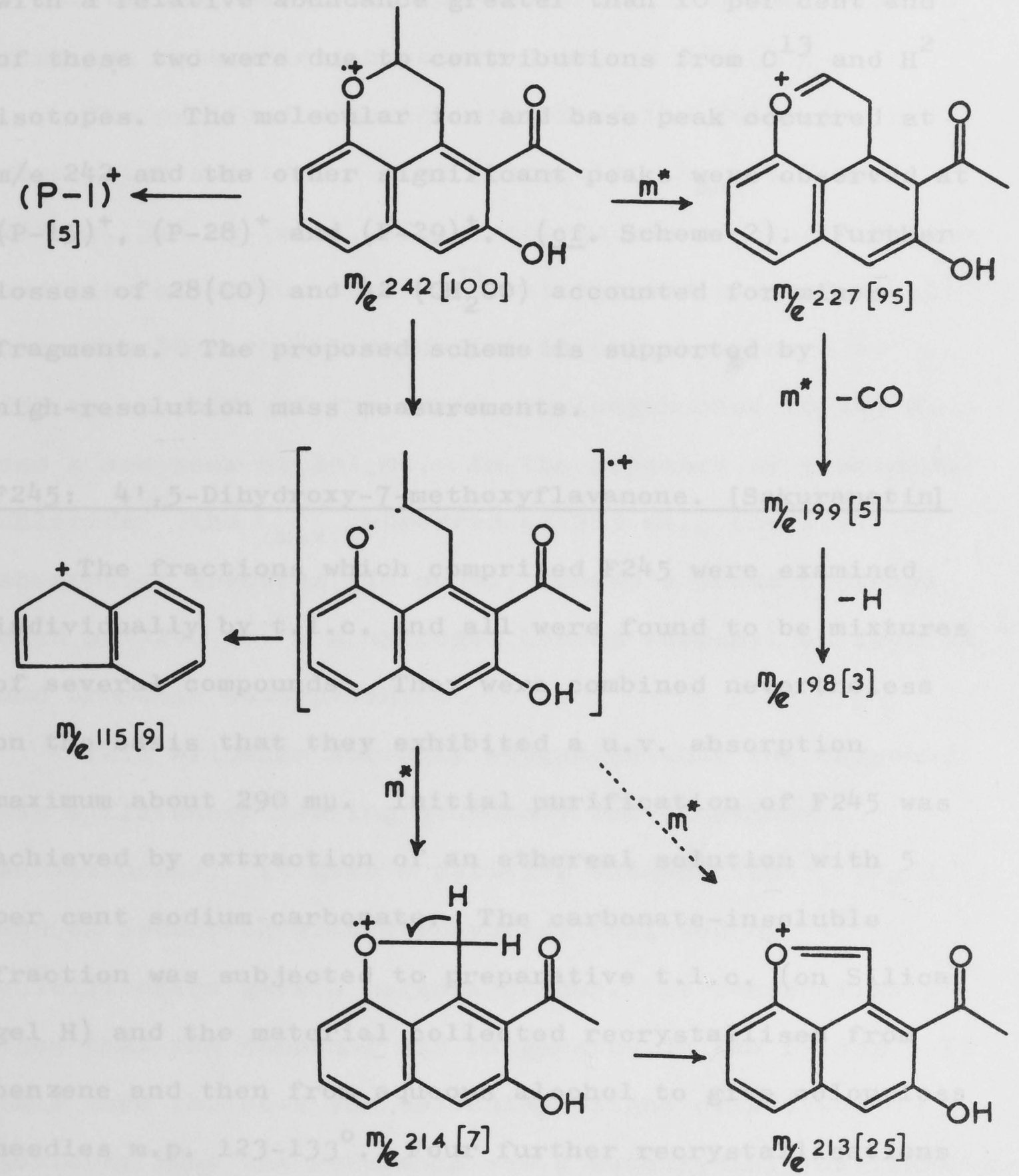
XIV



XIVa

The sodium salt of D-β-Hydroxybutyric acid was known to have a molecular rotation $[M]_D = -26^{o}$ [39] so the only remaining problem was to determine the optimum conditions for the ozonolysis and work-up procedures in order that the reaction could be carried out on a preparative scale. However, while this work was in progress we were informed by Duewell[32] that he had proceeded along similar lines and had isolated the (+)-form of β-Hydroxybutyric acid. On this basis it is concluded that the asymmetric centre at C-2 of xanthorrhoeol has the S configuration[40] as in (XIV).

When examined by low-resolution mass spectrometry, xanthorrhoeol exhibited a surprisingly simple spectrum. This probably accounts for the fact that it has not been mentioned in the literature. Only five peaks appeared


(P-1)+
[5]
m/e 242 [100]
m*
m/e 227 [95]
m* -CO
m/e 199 [5]
-H
m/e 198 [3]
m/e 115 [9]
m*
m*
m/e 214 [7]
m/e 213 [25]
OH
H

SCHEME 2

with a relative abundance greater than 10 per cent and of these two were due to contributions from C^{13} and H^2 isotopes. The molecular ion and base peak occurred at m/e 242 and the other significant peaks were observed at $(P-15)^+$, $(P-28)^+$ and $(P-29)^+$. (cf. Scheme 2). Further losses of 28(CO) and 42 (CH_2CO) accounted for minor fragments. The proposed scheme is supported by high-resolution mass measurements.

F245: 4',5-Dihydroxy-7-methoxyflavanone. [Sakuranetin]

The fractions which comprised F245 were examined individually by t.l.c. and all were found to be mixtures of several compounds. They were combined nevertheless on the basis that they exhibited a u.v. absorption maximum about 290 mμ. Initial purification of F245 was achieved by extraction of an ethereal solution with 5 per cent sodium carbonate. The carbonate-insoluble fraction was subjected to preparative t.l.c. (on Silica gel H) and the material collected recrystallised from benzene and then from aqueous alcohol to give colourless needles m.p. 123-133°. Four further recrystallisations (alternately from each solvent) failed to improve the m.p. beyond 131-135°. T.l.c., with a variety of solvent systems, indicated that only one compound was present.

A low voltage mass spectrum supported this and revealed that the molecular ion occurred at m/e 286.

The purified material exhibited the following u.v. absorption spectrum: F245 (Ethanol): $\lambda_{max.}$ 227 mμ, 288 mμ, 325 mμ (sh.). The spectrum was unchanged by the addition of sodium acetate[35] but the addition of alkali*[33] caused a 50 per cent reduction in the absorbance at 288 mμ, a shift of the short wavelength band to 245 mμ and a new peak at 361 mμ. In the presence of aluminium chloride[34] the $\lambda_{max.}$ appeared at 309 mμ. The i.r. absorption spectrum (in nujol) showed a broad hydroxyl band at 3240 cm^{-1}, a hydrogen bonded carbonyl at 1640 cm^{-1} and aromatic absorption.

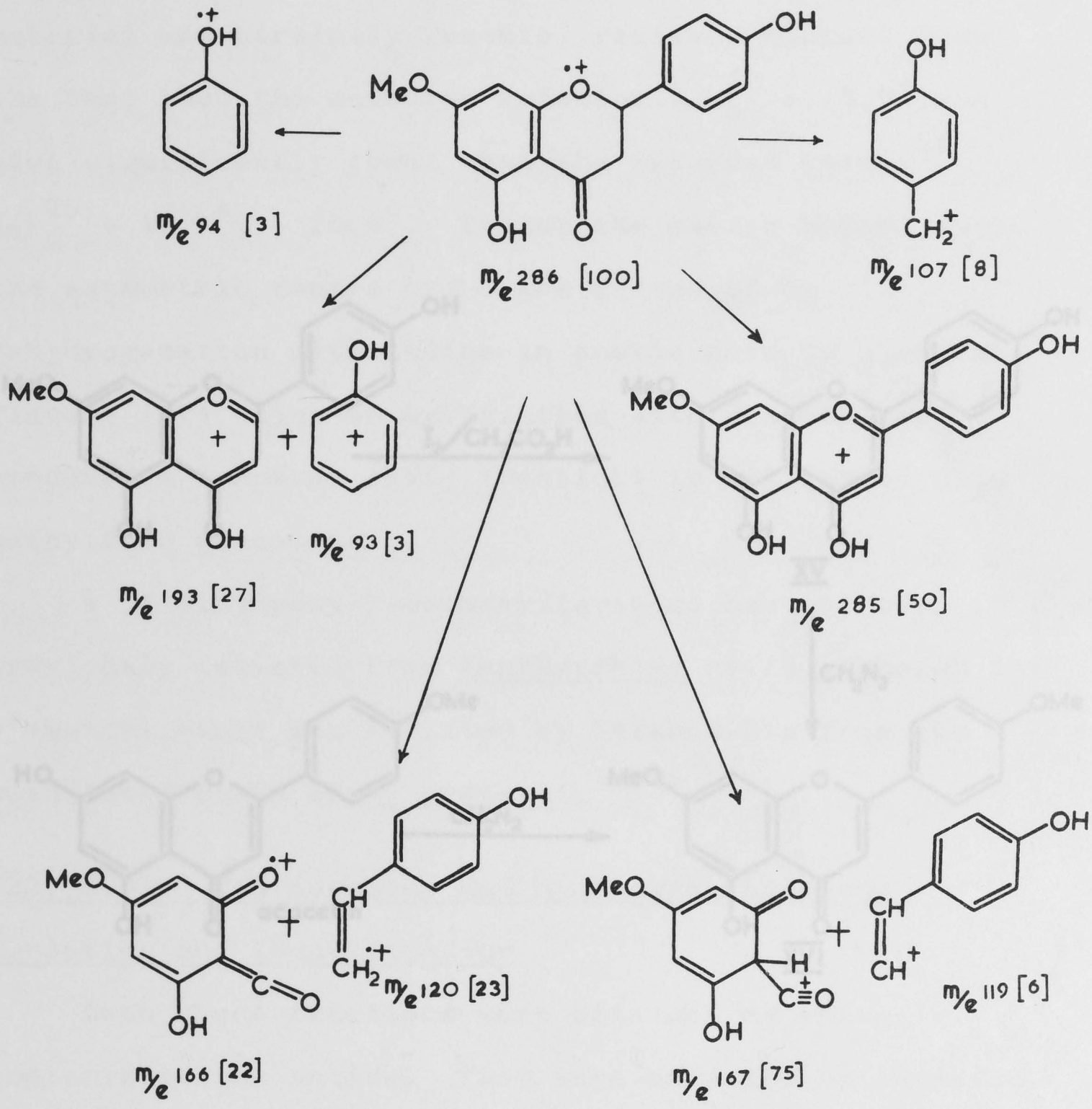
This evidence strongly suggested that the compound was a flavanone bearing 5-hydroxy and 7-methoxy substituents. It gave a positive Shinoda test[36] (Mg/HCl) and on reduction with borohydride followed by acidification gave a magenta colour. It seemed likely then that the compound was oxygenated in the B-ring. If this were the case the molecular weight of 286 would indicate that the substance was a methoxydihydroxyflavanone of formula $C_{16}H_{14}O_5$. This was confirmed by a high resolution mass measurement and an analysis. (Found: C, 67.1; H, 4.77; O, 28.13. Calc. for $C_{16}H_{14}O_5$: C, 67.1;

*i.e. 2N NaOH

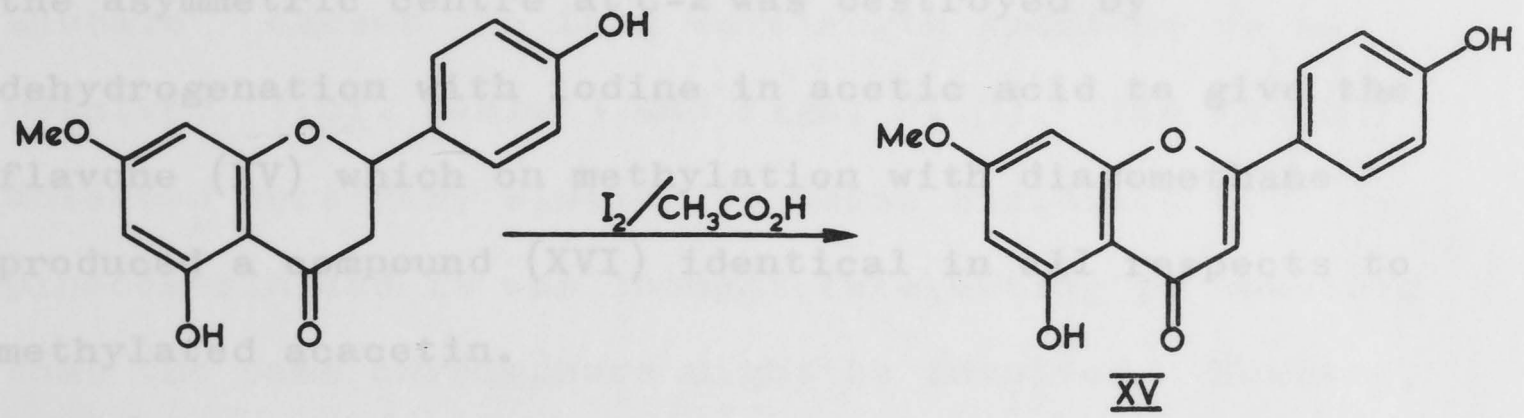
H, 4.9; O, 28.0). The mass spectrum of the compound showed a typical flavanone breakdown pattern and established that the additional hydroxyl group was in fact located in the B-ring (Scheme 3).

The 60 Mc./sec. p.m.r. spectrum in deuterochloroform was in agreement with that expected for sakuranetin, i.e. 4',5-dihydroxy-7-methoxyflavanone. The presence of the B-ring hydroxy group in the para position was clear from the A_2B_2 aromatic system which appeared essentially as a four-peak pattern. The protons on C-2' and C-6' were centred at τ 2.60, those on C-3' and C-5' at τ3.05 and the ortho coupling constant was about 9 c/s. The A-ring C-6, C-8 protons were observed as a sharp singlet at τ3.85. Aliphatic protons were accounted for by the multiplet at τ7.0 (2H) and the quartet centred at τ4.57 (1H)($J_{2,3a}$ = 11.5 c/s; $J_{2,3e}$ = 4.5 c/s; $J_{3e,3a}$ = 17.0 c/s). At the edge of the quartet, at τ4.35, the C-4' phenolic proton occurred, while the other phenolic proton, being hydrogen bonded, appeared at -τ2.50. The remaining three protons of the spectrum were due to the C-7 methoxy group and appeared as a sharp singlet at τ6.12.

Although there seemed little doubt that the compound had been correctly characterised the melting point 131-135°


m/e 94 [3]
m/e 286 [100]
m/e 107 [8]
m/e 93 [3]
m/e 193 [27]
m/e 285 [50]
m/e 120 [23]
m/e 166 [22]
m/e 119 [6]
m/e 167 [75]

SCHEME 3


MeO
OH
O
OH
O
I_2/CH_3CO_2H
MeO
OH
O
OH
O
XV
CH_2N_2
HO
OMe
O
OH
O
acacetin
CH_2N_2
MeO
OMe
O
OH
O
XVI

was significantly lower than the published value[41] m.p. 150°. The only plausible explanation, namely that the material was partially racemic, received support from the fact that the measured rotation $[\alpha]_D^{20} = -8.9°$ was also significantly lower than the recorded values[41] $[\alpha]_D^{20} + 10.7°$, $- 10.4°$. To put the matter beyond doubt the asymmetric centre at C-2 was destroyed by dehydrogenation with iodine in acetic acid to give the flavone (XV) which on methylation with diazomethane produced a compound (XVI) identical in all respects to methylated acacetin.

4',5-dihydroxy-7-methoxyflavanone has not been previously isolated from Xanthorrhoea resin although its 4'-methyl ether was isolated by Salahud-Din from the methylated resin of X. preissii.[17,28]

F22, F401: Xanthorrhone and Hydroxyxanthorrhone

Isolation and Identification

Both these fractions were obtained as white semicrystalline solids. They were purified by repeated recrystallisation from benzene (F22), or chloroform (F401), to give colourless needles m.p. 193-196° and m.p. 194-196° respectively.

Initially, attention was drawn to the substances by the observation that they both exhibited flavanone-type

u.v. absorption spectra with a $\lambda_{max.}$ wavelength greater than 290 mμ. (Di- and tri-hydroxyflavanones such as pinocembrin and naringenin have a $\lambda_{max.}$ at 290 mμ and the methyl ethers of these have a $\lambda_{max.}$ at shorter wavelengths). Bathochromic shifts were observed on the addition of alkali[33] or aluminium chloride,[34] and sodium acetate[35] caused the long wavelength shoulder to be resolved. (cf. Table 3 and Figs. 2, 3). The shifts observed were very similar to those exhibited by pinocembrin and it was thought interesting to consider that the same chromophore might be involved. However, when a value of $\varepsilon_{max.}$ 17,850 was used for the pinocembrin chromophore the apparent molecular weights of F22 and F401 were calculated at ca. 570 and ca. 600 respectively, indicating that a dimeric structure might be involved. Osmometric measurements supported this giving an approximate molecular weight for F401 of 520-560.

XANTHORRHONE

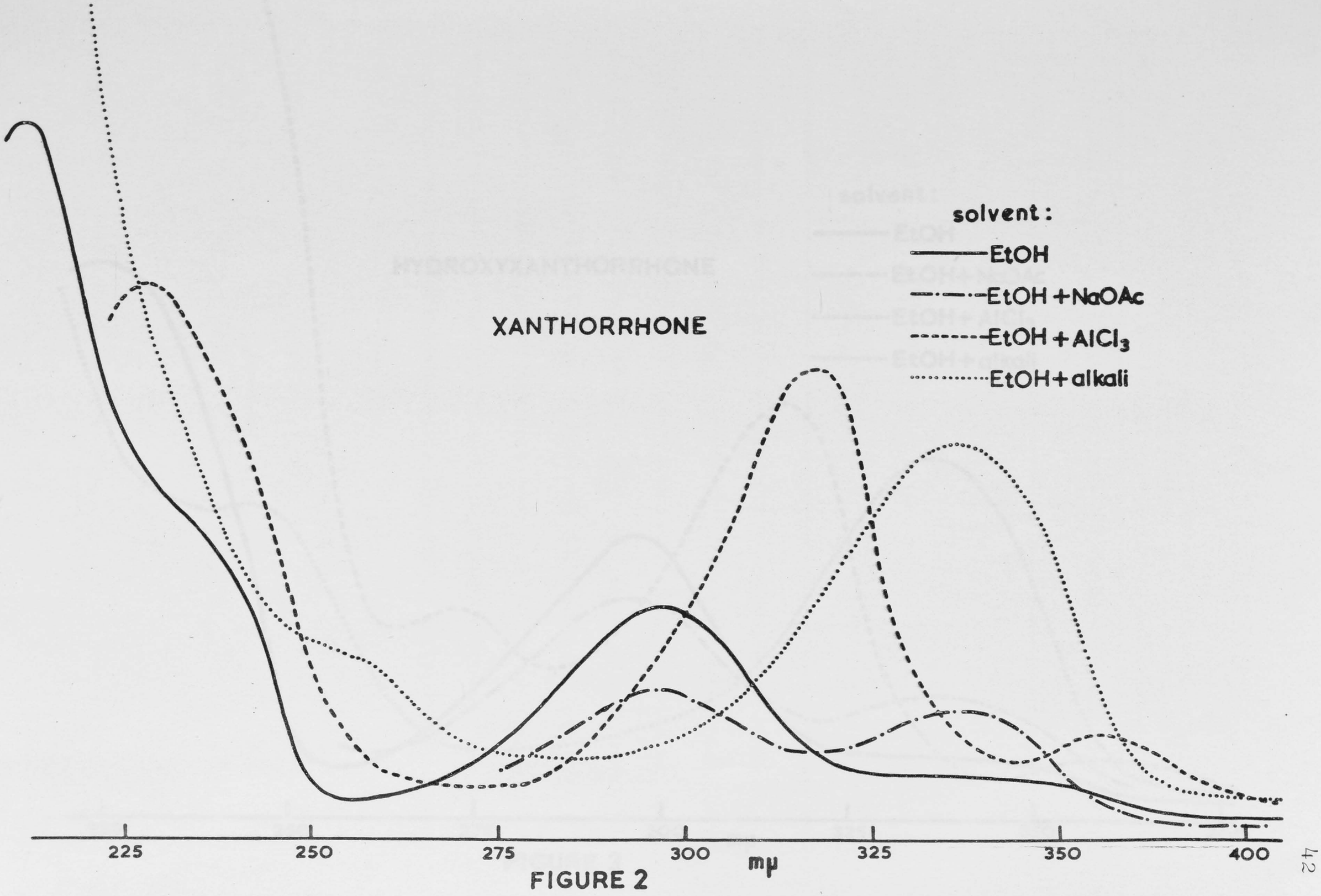


FIGURE 2

HYDROXYXANTHORRHONE

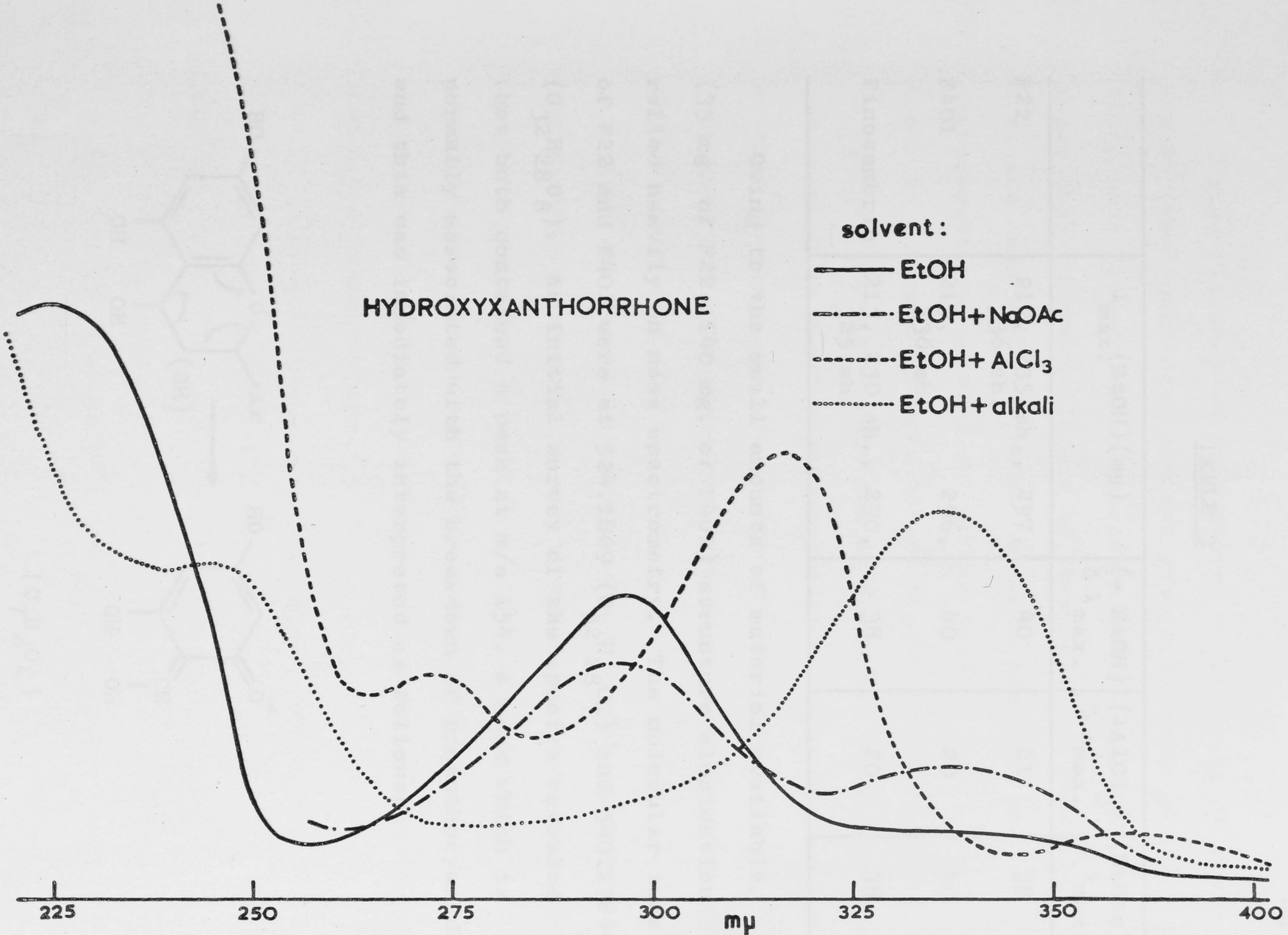
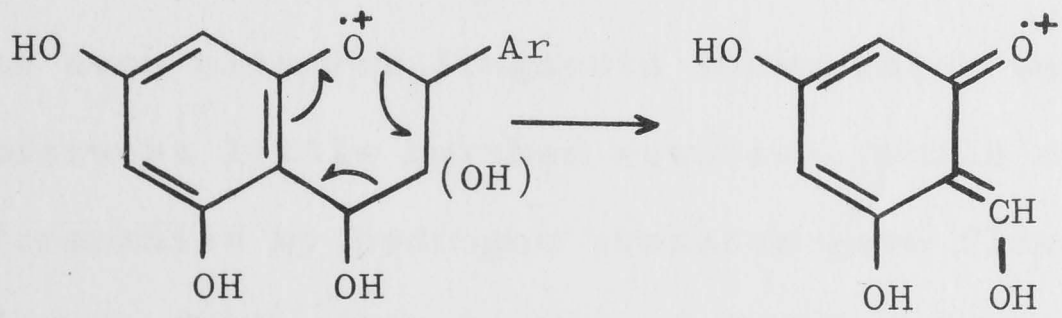


FIGURE 3

TABLE 3

	$\lambda_{max.}$ (EtOH) (mμ)	(+ NaOH) $\Delta \lambda_{max.}$	(+$AlCl_3$) $\Delta \lambda_{max.}$	(NaOAc) $\Delta \lambda_{max.}$
F22	212, 235 sh., 297, 336 sh.	40	21	39
F401	215, 296, 336 sh.	40	20	40
Pinocembrin	213, 230 sh., 290, 325 sh.	38	20	38

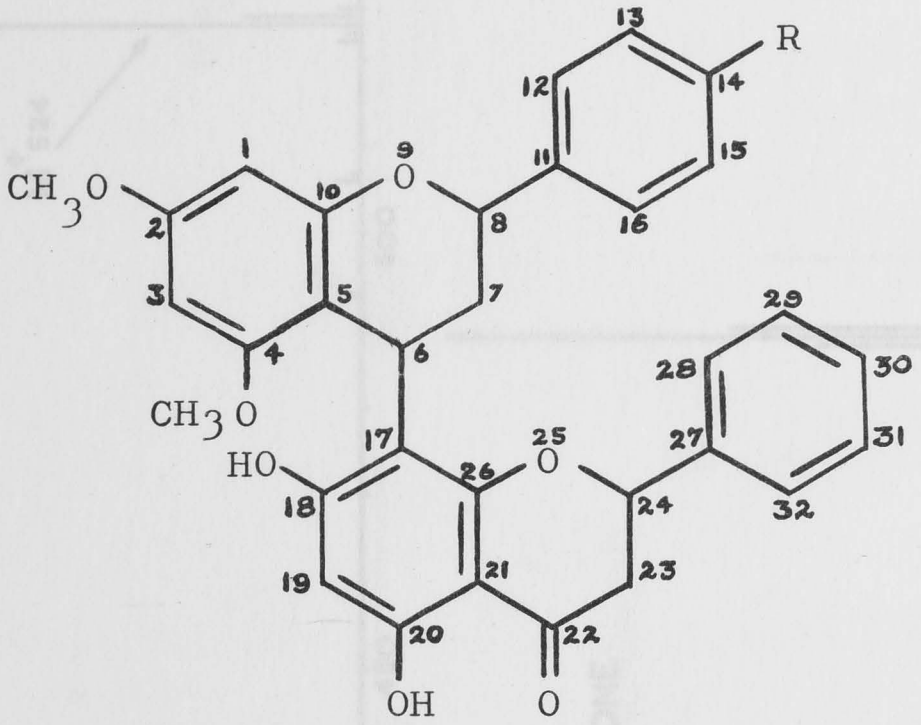
Owing to the small amounts of material available (35 mg. of F22; 140 mg. of F401) structure elucidation relied heavily on mass spectrometry. The molecular ions of F22 and F401 were at 524.1869 ($C_{32}H_{28}O_7$) and 540.1784 ($C_{32}H_{28}O_8$). An initial survey of the spectra revealed that both contained a peak at m/e 154, a peak which is normally associated with the breakdown of proanthocyanidins, and this was immediately interpreted as follows:



[$C_7H_6O_4$]

Using flavan-4-ol derivatives as one unit of the dimer, (the substances did not give a proanthocyanidin test) many systems were devised, however, it proved impossible to reconcile any of them with the observed u.v. and mass spectral data. When an accurate mass measurement was carried out on the peak it was found to occur as a singlet at m/e 154.0626 and this corresponded to $C_8H_{10}O_3$ not $C_7H_6O_4$ and the fragment was then correctly interpreted as dimethylphloroglucinol. This was unusual as this fragment was not typical of any known simple flavonoid and must therefore have been characteristic of the molecule as a whole.

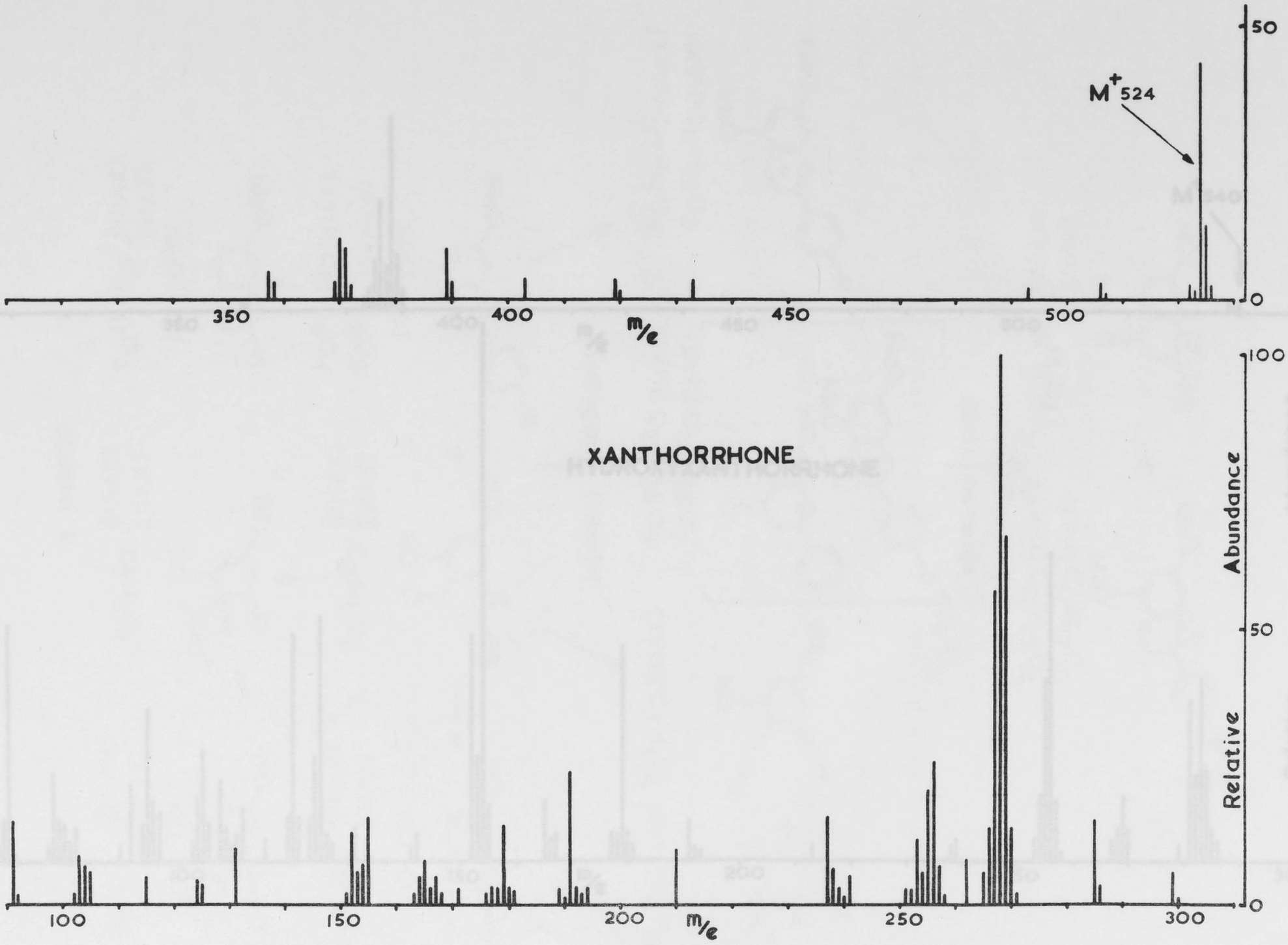
With the need to incorporate a flavanol unit into the molecules removed, it was then possible to postulate new structures for F22 and F401. The most likely structures to emerge were (XVII) and (XVIII), respectively, and the substances were named xanthorrhone and hydroxyxanthorrhone.

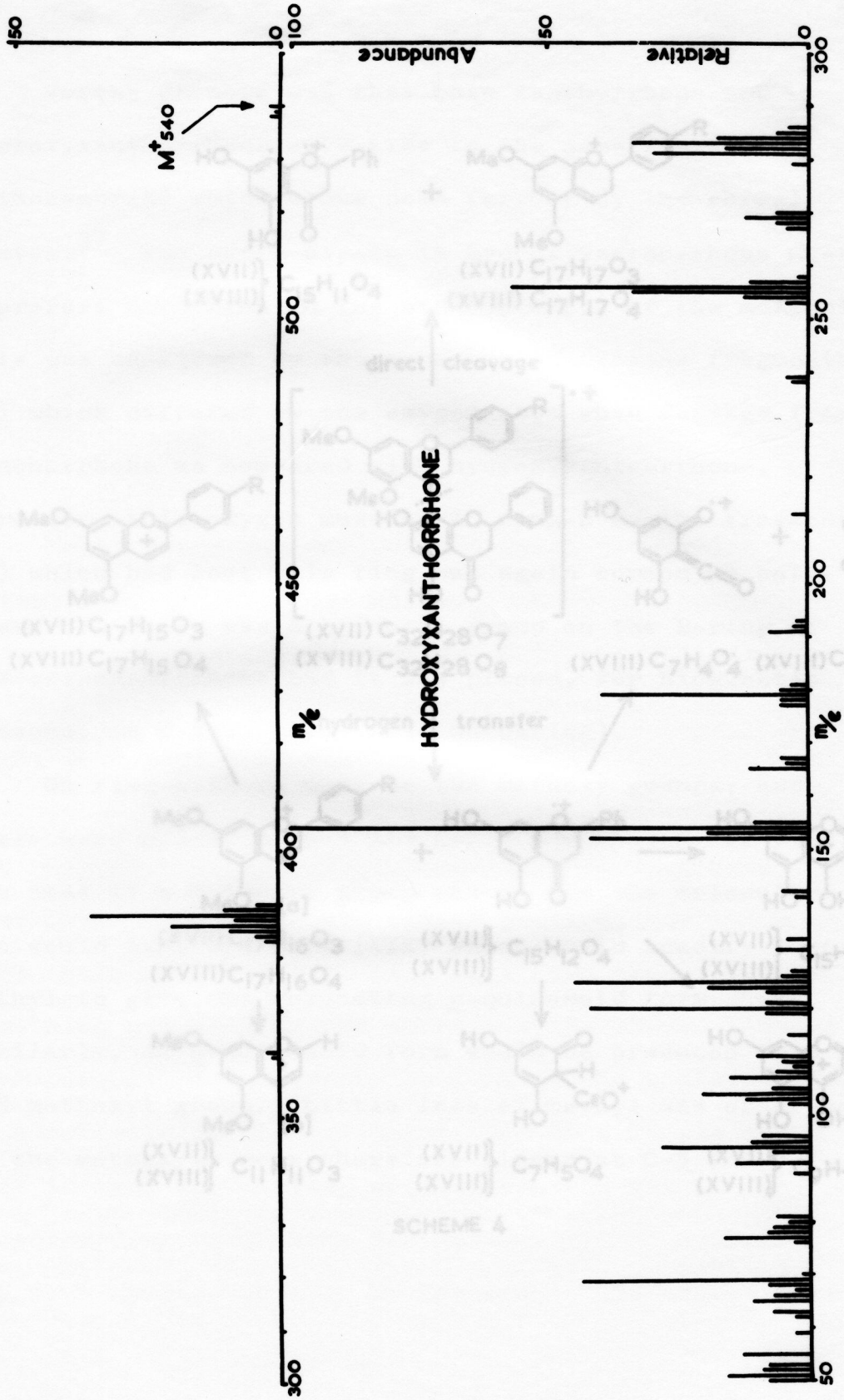


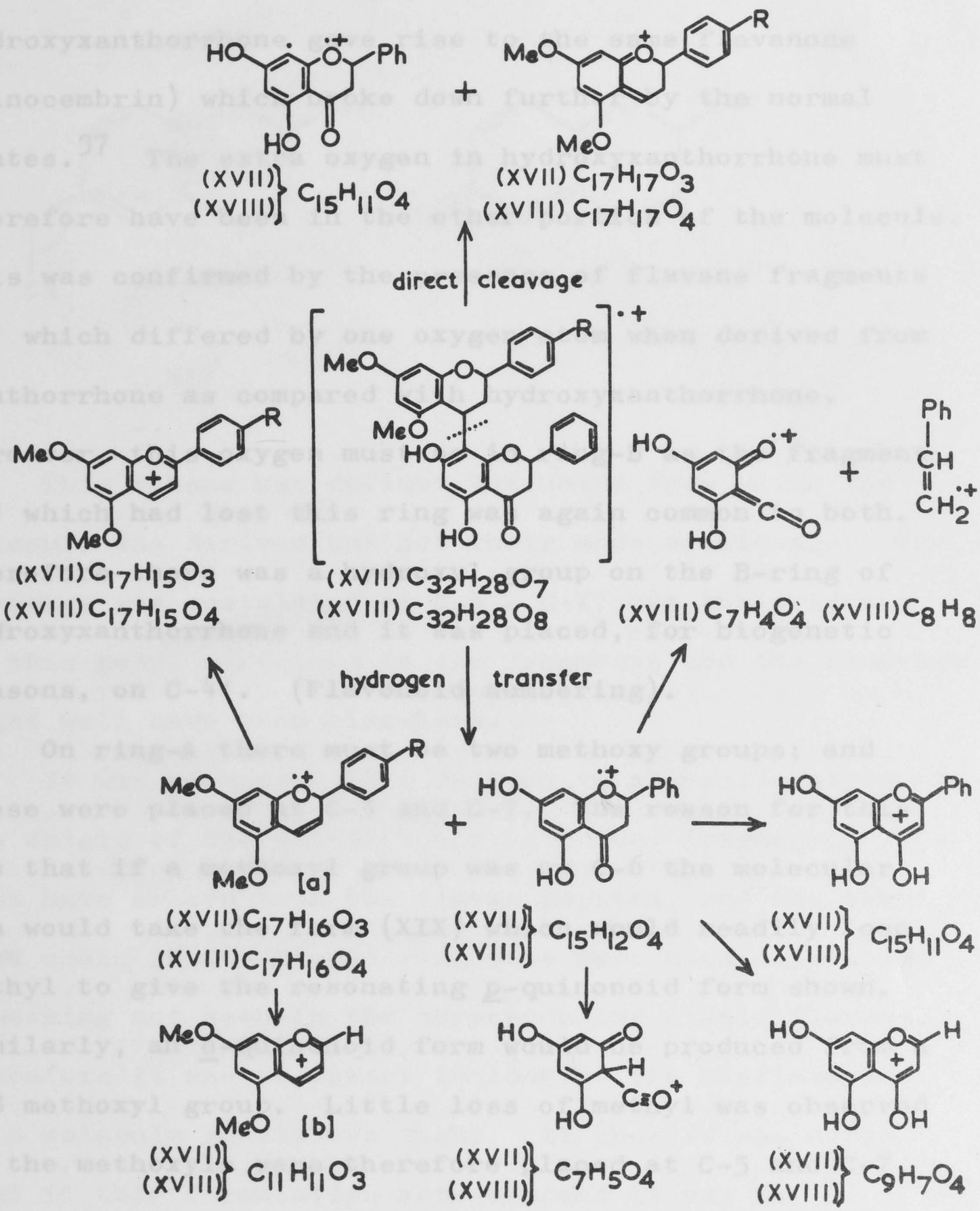
XVII; R = H

XVIII; R = OH

Schemes 4 and 5 represent rationalised interpretations of the mass spectra. All the formulae given are supported by accurate mass measurements. Scheme 4 shows the results of splitting of the C-6 - C-17 bond with consequent relief of steric strain from the heavily crowded region of the molecule. Direct cleavage gave the even electron fragments shown which were stable and underwent little further reaction, while cleavage accompanied by hydrogen transfer gave flavanone and flavene fragments capable of further fragmentation.


50
M+524
0
350
400
450
500
m/e
XANTHORRHONE
100
50
0
Relative Abundance
100
150
200
250
300
m/e

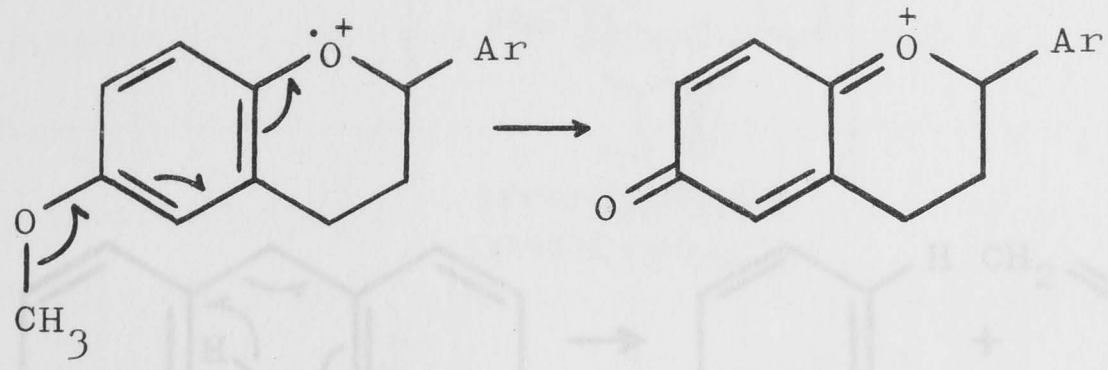

HYDROXYXANTHORRHONE
M+ 540
Abundance
Relative
m/e
m/e


(XVII) C15H11O4
(XVIII)
(XVII) C17H17O3
(XVIII) C17H17O4
direct cleavage
(XVII) C17H15O3
(XVIII) C17H15O4
(XVII) C32H28O7
(XVIII) C32H28O8
(XVIII) C7H4O4
(XVIII) C8H8
hydrogen transfer
[a]
(XVII) C17H16O3
(XVIII) C17H16O4
(XVII) C15H12O4
(XVIII)
(XVII) C15H11O4
(XVIII)
[b]
(XVII) C11H11O3
(XVIII)
(XVII) C7H5O4
(XVIII)
(XVII) C9H7O4
(XVIII)

SCHEME 4

Worthy of note was that both xanthorrhone and hydroxyxanthorrhone gave rise to the same flavanone (pinocembrin) which broke down further by the normal routes.[37] The extra oxygen in hydroxyxanthorrhone must therefore have been in the other portion of the molecule. This was confirmed by the presence of flavene fragments (a) which differed by one oxygen atom when derived from xanthorrhone as compared with hydroxyxanthorrhone. Moreover, this oxygen must be in ring-B as the fragment (b) which had lost this ring was again common to both. Therefore there was a hydroxyl group on the B-ring of hydroxyxanthorrhone and it was placed, for biogenetic reasons, on C-4'. (Flavonoid numbering).

On ring-A there must be two methoxy groups; and these were placed at C-5 and C-7. The reason for this was that if a methoxyl group was on C-6 the molecular ion would take the form (XIX) which would readily lose methyl to give the resonating p-quinonoid form shown. Similarly, an o-quinonoid form would be produced from a C-8 methoxyl group. Little loss of methyl was observed so the methoxyls were therefore placed at C-5 and C-7.

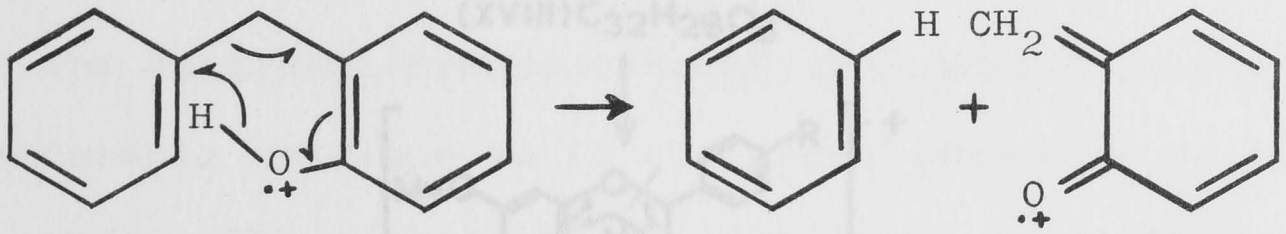


XIX

This scheme has defined the units from which the molecule was derived but not their mode of linkage. The cleavage was postulated at C-6 - C-17 but the evidence to this point pertained to the fragments and the cleavage might well have been elsewhere.

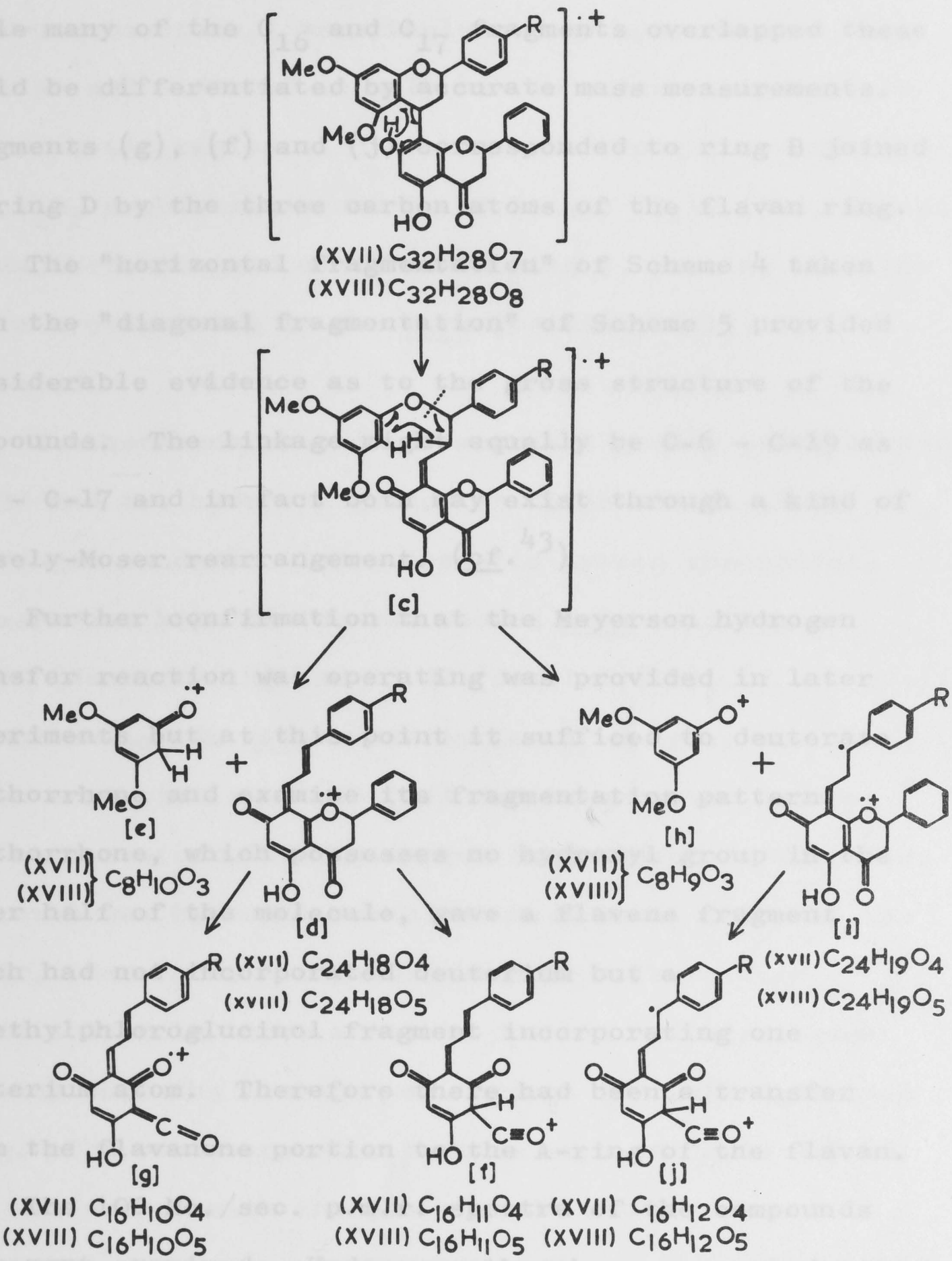
It was necessary then to turn to a consideration of the origin of the dimethylphloroglucinol fragment. This must have arisen from the flavan portion, and the alkyl side chain of the flavan ring must have been displaced; something not seen in the corresponding simple flavans. Therefore it was necessary to look at the bisflavonoid as a molecule in its own right. At once it was noted that if this formulation were correct it was an o-hydroxydiphenylmethane derivative. These compounds had been studied earlier by Meyerson[42] who showed that

their characteristic cleavage was a hydrogen transfer with displacement of the alkyl chain.



When this was applied to the xanthorrhones the molecular ion took the form (c) (Scheme 5). When this was followed by a direct cleavage the C_{24} fragments were produced, and further, when the more prominent cleavage with hydrogen transfer took place, dimethyl phloroglucinol arose as a normal fragment. This could only have happened if the linkage was as shown.

The fragments (d) and (i) underwent reverse Diels-Alder reactions to yield the C_{16} products, all of which contained ring B as they differed by one oxygen when derived from xanthorrhone as compared with hydroxyxanthorrhone. It was therefore a considerable advantage to have two compounds to compare. It was noted that


(XVII) C32H28O7
(XVIII) C32H28O8
[c]
[e]
(XVII) } C8H10O3
(XVIII) }
[d]
[h]
(XVII) } C8H9O3
(XVIII) }
[i]
[g]
(XVII) C16H10O4
(XVIII) C16H10O5
(XVII) C24H18O4
(XVIII) C24H18O5
[f]
(XVII) C16H11O4
(XVIII) C16H11O5
(XVII) C24H19O4
(XVIII) C24H19O5
[j]
(XVII) C16H12O4
(XVIII) C16H12O5

SCHEME 5

while many of the C_{16} and C_{17} fragments overlapped these could be differentiated by accurate mass measurements. Fragments (g), (f) and (j) corresponded to ring B joined to ring D by the three carbon atoms of the flavan ring.

The "horizontal fragmentation" of Scheme 4 taken with the "diagonal fragmentation" of Scheme 5 provided considerable evidence as to the gross structure of the compounds. The linkage might equally be C-6 - C-19 as C-6 - C-17 and in fact both may exist through a kind of Wessely-Moser rearrangement. (cf.[43])

Further confirmation that the Meyerson hydrogen transfer reaction was operating was provided in later experiments but at this point it sufficed to deuterate xanthorrhone and examine its fragmentation pattern. Xanthorrhone, which possesses no hydroxyl group in the upper half of the molecule, gave a flavene fragment which had not incorporated deuterium but a dimethylphloroglucinol fragment incorporating one deuterium atom. Therefore there had been a transfer from the flavanone portion to the A-ring of the flavan.

The 100 Mc./sec. p.m.r. spectra of the compounds were next examined. Hydroxyxanthorrhone presented some difficulties due to its low solubility in chloroform (ca. 0.9 mg./ml.) and it was necessary to run the spectrum

in deuteroacetone. Nevertheless, the spectra were very similar and differed in only one important respect. In the aromatic region of hydroxyxanthorrhone there were two A_2B_2 doublets centred at τ2.79 and τ3.19 (4H), absent in xanthorrhone and ascribed to ring B, proving that the extra hydroxyl was in the para-position (C-14) as expected.

Xanthorrhone showed two methoxyl groups at τ6.25 and τ6.32 and two hydroxyl groups at τ3.38 and τ - 2.63 (hydrogen bonded). Three aromatic proton absorptions appeared at high field; two showed meta coupling at τ3.80 (doublet)(1H) and τ3.92 (doublet)(1H), (J = 2 c/s), establishing the meta substitution of ring A, and the other a singlet at τ4.08 (1H) due to a proton between two carbon atoms bearing oxygen substituents. Since one hydroxyl was hydrogen bonded it must be at C-20 and the other, therefore, was placed at C-18. There were definitely only three aromatic protons in this region showing that the linkage was through the phloroglucinol ring. The other aromatic protons were at τ2.60 - 2.82.

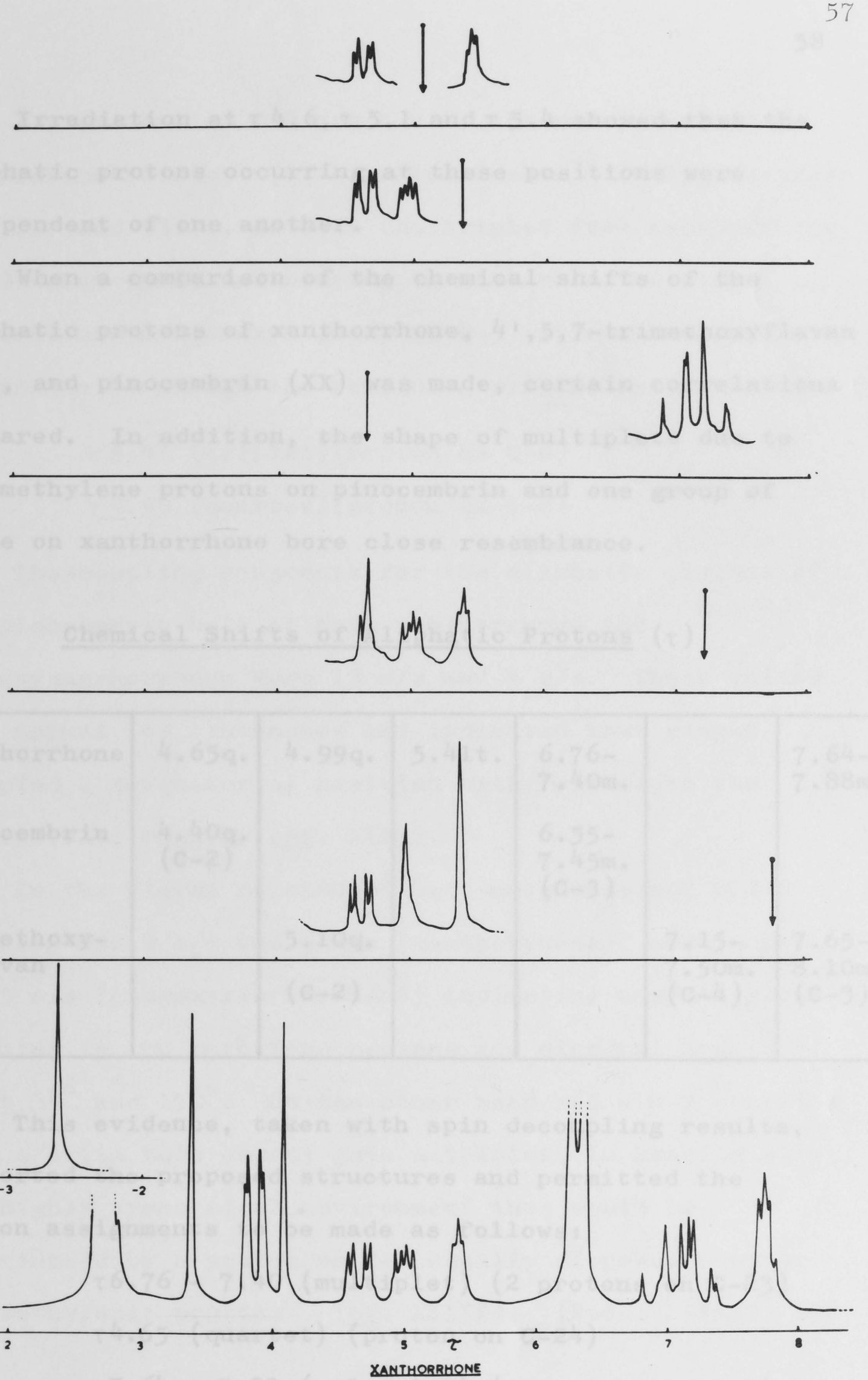
In accord with the postulated linkage seven aliphatic protons were observed. They appeared at τ 4.65 (quartet)(1H) (J_1 = 13 c/s; J_2 = 4 c/s), τ 4.99 (quartet)(1H) (J_1 = 9 c/s; J_2 = 5 c/s), τ 5.41 (triplet)(1H)

(J = 4 c/s), τ6.76 - 7.40 (mult.) (2H) and τ7.64 - 7.88 (mult.)(2H).

Hydroxyxanthorrhone (in deuteroacetone) showed methoxyl groups at τ6.30 and τ6.42 and aliphatic protons at τ4.49 (quartet) (1H)(J_1 = 13 c/s; J_2 = 4 c/s), τ4.75 (quartet)(1H)(J_1 = 10 c/s; J_2 = 5 c/s), τ5.40 (triplet) (1H)(J = 4 c/s), τ6.7 - 7.4 (mult.)(2H) and a multiplet about τ7.8 which merged with the acetone signal. Hydroxyl protons absorbed at τ7.0(under the multiplet)and at - τ2.62 (hydrogen bonded). The aromatic protons were accounted for by the multiplet at τ2.35 - 2.70 (5H),A_2B_2 doublets at τ2.79 and τ3.19 (4H) and an unsymmetrical doublet, integrating for 3 protons, due to the A-ring meta protons (τ3.98) and the corresponding proton from the flavanone unit (τ3.93).

Xanthorrhone was subjected to spin-decoupling experiments (cf. Fig.4). Irradiation at τ7.7 caused the quarted at τ4.99 to collapse to a broad singlet, the triplet at τ5.41 to become a sharp singlet and left the quarted at τ4.65 unchanged. Thus a - CH - CH_2 - CH - system was indicated.

Irradiation at τ7.09 made the quartet at τ4.65 collapse to a highly unsymmetrical doublet, whilst irradiation at τ4.6 produced a quartet from the multiplet at τ7.09. Thus a - CH - CH_2 - system was indicated.



XANTHORRHONE

FIGURE 4

Irradiation at τ 4.6, τ 5.1 and τ 5.4 showed that the aliphatic protons occurring at these positions were independent of one another.

When a comparison of the chemical shifts of the aliphatic protons of xanthorrhone, 4',5,7-trimethoxyflavan (XI), and pinocembrin (XX) was made, certain correlations appeared. In addition, the shape of multiplets due to the methylene protons on pinocembrin and one group of those on xanthorrhone bore close resemblance.

Chemical Shifts of Aliphatic Protons (τ)

Xanthorrhone	4.65q.	4.99q.	5.41t.	6.76-7.40m.		7.64-7.88m.
Pinocembrin	4.40q. (C-2)			6.55-7.45m. (C-3)		
Trimethoxy-flavan		5.10q. (C-2)			7.15-7.50m. (C-4)	7.65-8.10m. (C-3)

This evidence, taken with spin decoupling results, supported the proposed structures and permitted the proton assignments to be made as follows:

τ6.76 - 7.40 (multiplet) (2 protons on C-23)

τ4.65 (quartet) (proton on C-24)

τ7.64 - 7.88 (multiplet) (2 protons on C-7)

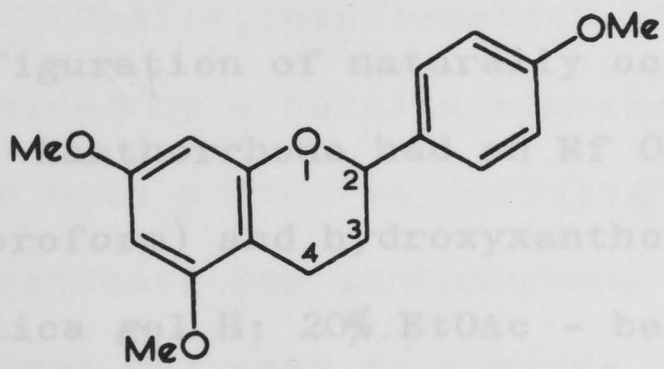
The protons at C-2 and C-4 of the model compound, leucorobinetinidin - (+) - gallocatechin[44] (XXI), occurred at τ5.57 and τ5.21, so with the triplet from xanthorrhone standing at τ5.41 and bearing in mind that the model compound has a hydroxyl group at C-3, it was thought reasonable to assign the remaining protons as:

τ 5.41 (triplet)(proton on C-6)

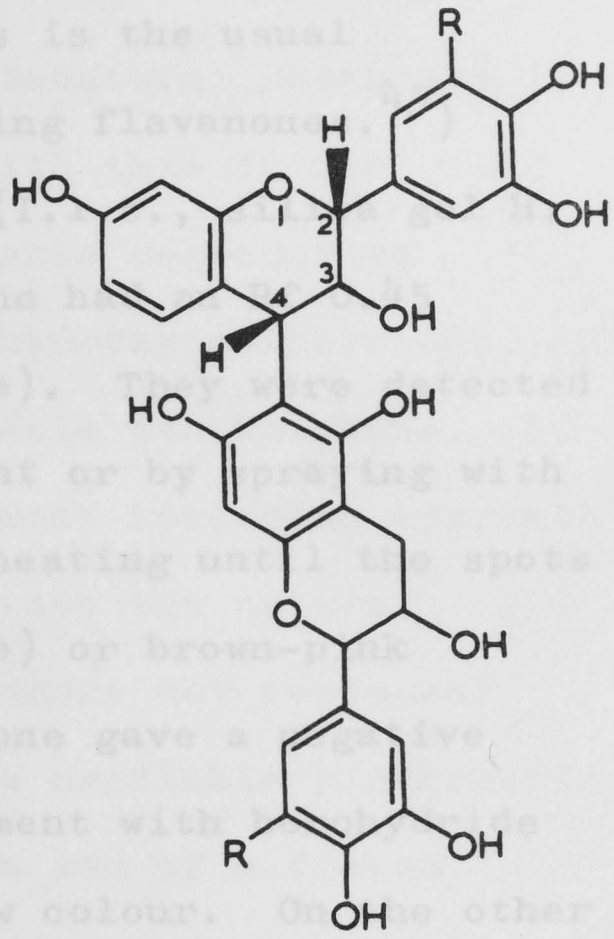
τ 4.99 (quartet)(proton on C-8)

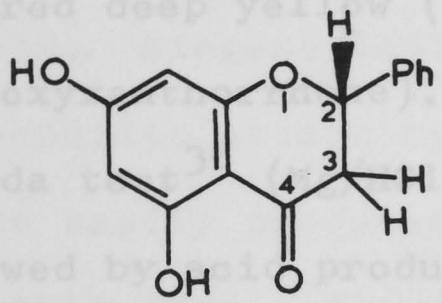
The coupling constants for the aliphatic protons of the pinocembrin half of both xanthorrhone and hydroxyxanthorrhone were 13 c/s and 4 c/s. These values were normal for flavanones and indicated that ring-E occupied a ψ-equatorial position with respect to the heterocyclic ring F. (cf. XXII).

In the flavan portion of both molecules H-7 - H-8 coupling was 9 c/s and 5 c/s (xanthorrhone), and 10 c/s and 5 c/s (hydroxyxanthorrhone) indicating unsymmetrical coupling to the methylene protons and dihedral angles of about 34^{o} and 170^{o}. On the other hand H-6 - H-7 coupling of 4 c/s (in both cases) gave a triplet, in keeping with the highly symmetrical environment that would be experienced by a proton on C-6 equally disposed towards C-7 methylenic protons. (cf. XXIII). (Note: (XXII) and



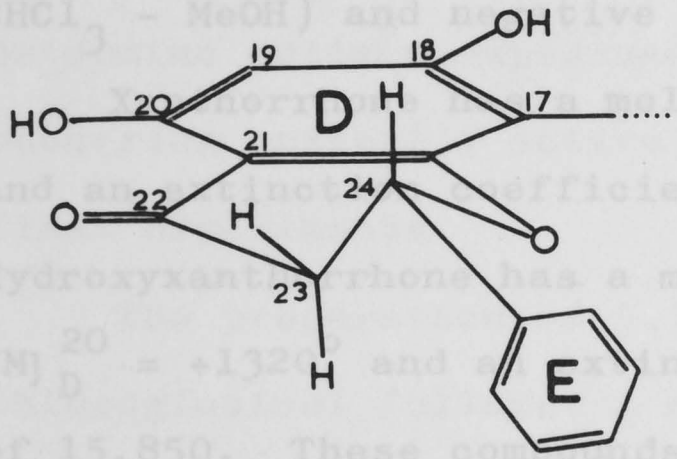
XI



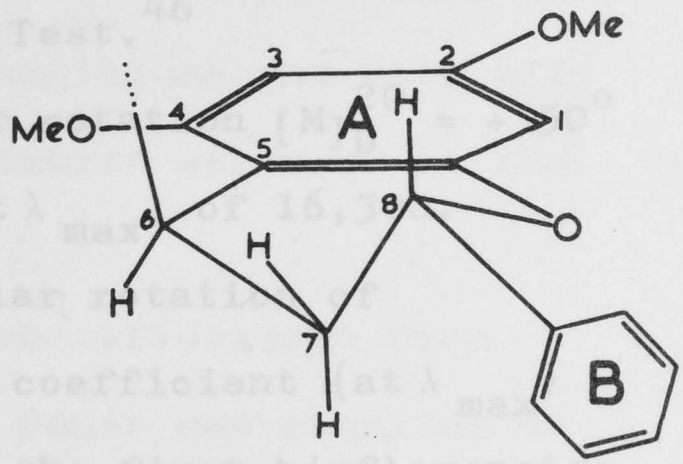


XX

XXI



XXII



XXIII

(XXIII) are drawn with the S configuration[40] at C-8 and C-24 purely on the basis that this is the usual configuration of naturally occurring flavanones.[45])

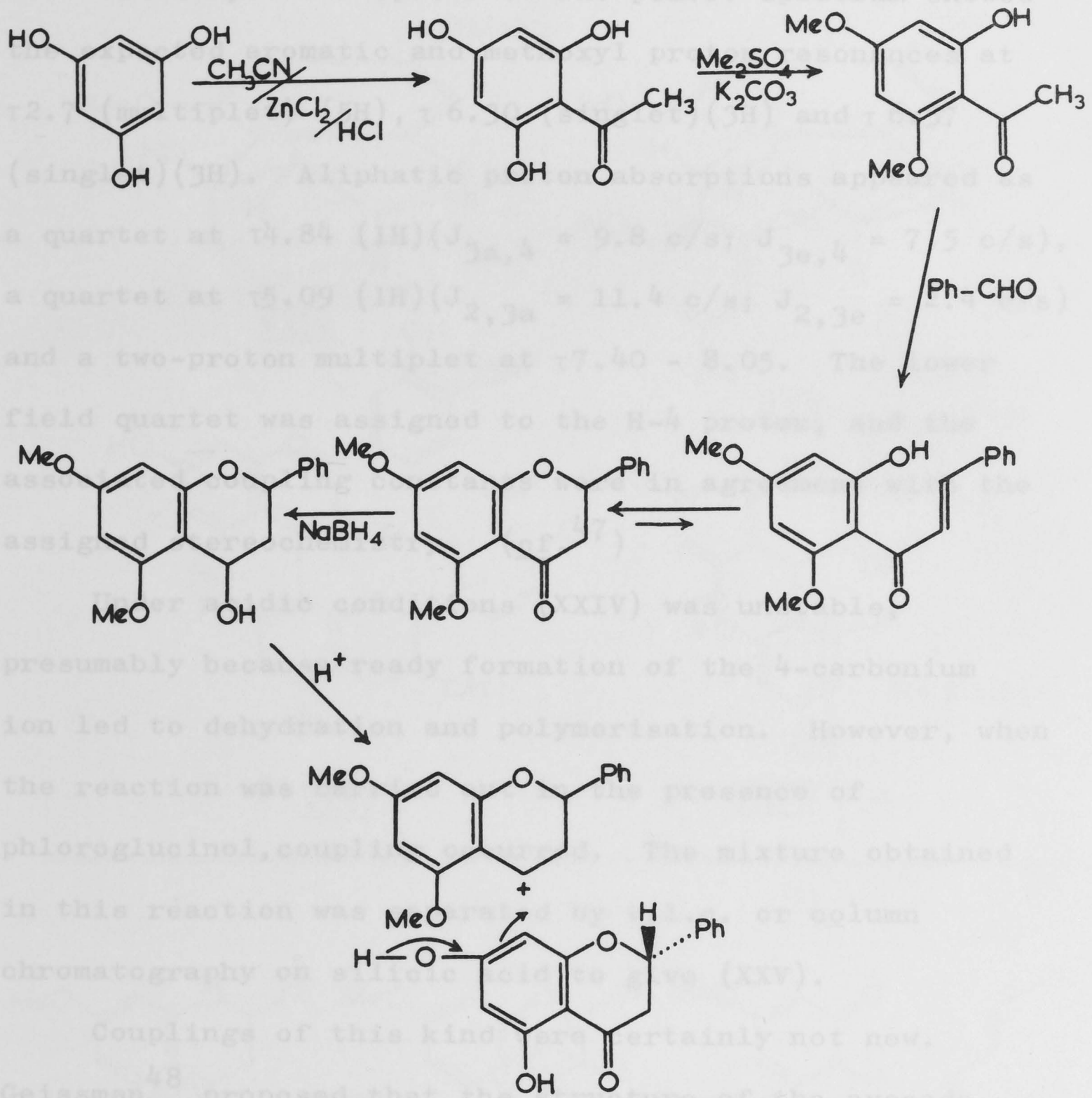
Xanthorrhone had an Rf 0.69 (T.l.c., silica gel H; chloroform) and hydroxyxanthorrhone had an Rf 0.45 (Silica gel H; 20% EtOAc - benzene). They were detected by their fluorescence in u.v. light or by spraying with concentrated sulphuric acid then heating until the spots appeared deep yellow (xanthorrhone) or brown-pink (hydroxyxanthorrhone). Xanthorrhone gave a negative Shinoda test[36] (Mg/HCl) and treatment with borohydride followed by acid produced a yellow colour. On the other hand hydroxyxanthorrhone gave the characteristic magenta colour produced by flavanones under these conditions. Both compounds gave a green ferric chloride test (in $CHCl_3$ - MeOH) and negative Gibbs Test.[46]

Xanthorrhone has a molecular rotation $[M]_D^{20} = +550^o$ and an extinction coefficient (at λ_{max}) of 16,300. Hydroxyxanthorrhone has a molecular rotation of $[M]_D^{20} = +1320^o$ and an extinction coefficient (at λ_{max}) of 15,850. These compounds were the first bisflavonoids to be isolated having the heterocyclic rings in different oxidation states.

Synthetic Evidence for the Structure of Xanthorrhone

Ideally, confirmation of a structural postulate is provided by a total synthesis. With this in mind, and also with a view to devising suitable degradative experiments for xanthorrhone and hydroxyxanthorrhone, an attempt was made to prepare synthetic xanthorrhone. It was considered that probably the most rewarding approach would come from an attempt to imitate the natural process. Biogenetically, these dimers may represent nucleophilic attack by the readily available pinocembrin on the easily produced 4-carbonium ion of a flavan (possibly from a flavan-4-ol) and for this reason the synthetic scheme drawn up relied on this reaction. (*cf.* Scheme 6). Phloroglucinol was used as a model compound for pinocembrin in preliminary experiments to determine optimum experimental conditions and naturally occurring optically active pinocembrin was used in the final experiments.

The preparation of 5,7-dimethoxyflavanone from phloroglucinol followed a normal route and requires no further comment. 5,7-Dimethoxyflavanone reacted with sodium borohydride in methanol to give the 2,4-*cis* alcohol (XXIV). This structural assignment was made

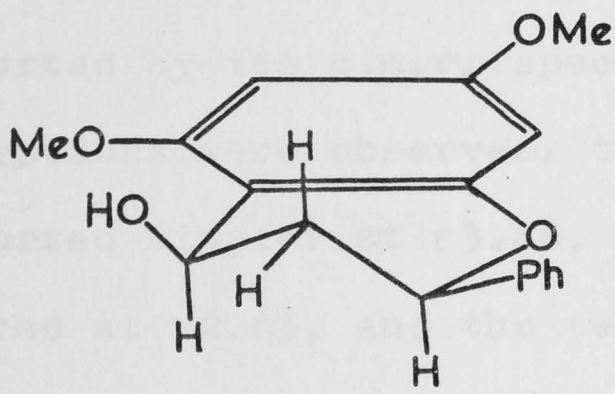

HO
OH
OH
CH3CN
ZnCl2
HCl
HO
OH
CH3
OH
Me2SO4
K2CO3
MeO
OH
CH3
MeO
Ph-CHO
MeO
O
Ph
MeO
OH
NaBH4
MeO
O
Ph
MeO
O
MeO
OH
Ph
MeO
H+
MeO
O
Ph
+
MeO
H
O
H
Ph
O
OH
O

SCHEME 6

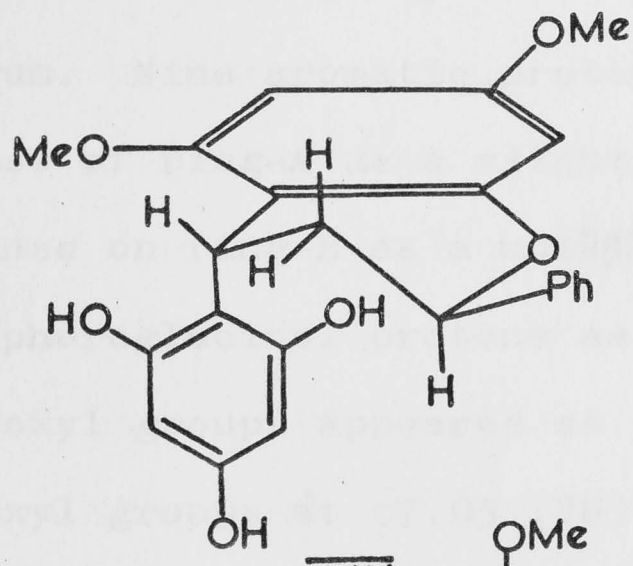
from an analysis of spectra. The p.m.r. spectrum showed the expected aromatic and methoxyl proton resonances at τ2.7 (multiplet) (5H), τ 6.30 (singlet)(3H) and τ 6.37 (singlet)(3H). Aliphatic proton absorptions appeared as a quartet at τ4.84 (1H)($J_{3a,4}$ = 9.8 c/s; $J_{3e,4}$ = 7.5 c/s), a quartet at τ5.09 (1H)($J_{2,3a}$ = 11.4 c/s; $J_{2,3e}$ = 2.4 c/s) and a two-proton multiplet at τ7.40 - 8.05. The lower field quartet was assigned to the H-4 proton, and the associated coupling constants were in agreement with the assigned stereochemistry. (cf.[47])

Under acidic conditions (XXIV) was unstable, presumably because ready formation of the 4-carbonium ion led to dehydration and polymerisation. However, when the reaction was carried out in the presence of phloroglucinol, coupling occurred. The mixture obtained in this reaction was separated by t.l.c. or column chromatography on silicic acid to give (XXV).

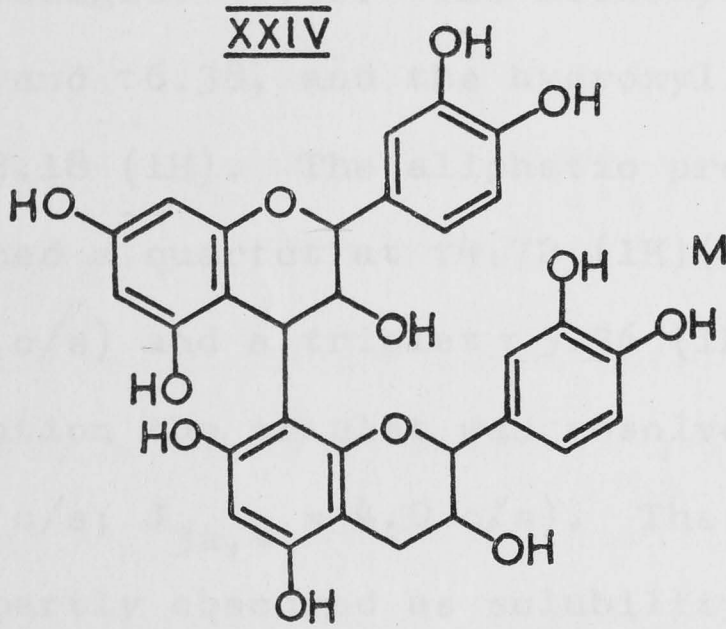
Couplings of this kind were certainly not new. Geissman[48] proposed that the structure of the avocado dimer was (XXVI) arising from the acid catalysed condensation between a flavan-3,4-diol and catechin. Later, he and Yoshimura[49] demonstrated the reaction by preparing (XXVII) and a derivative of (XXVI). This work has subsequently been confirmed by other workers.[50]



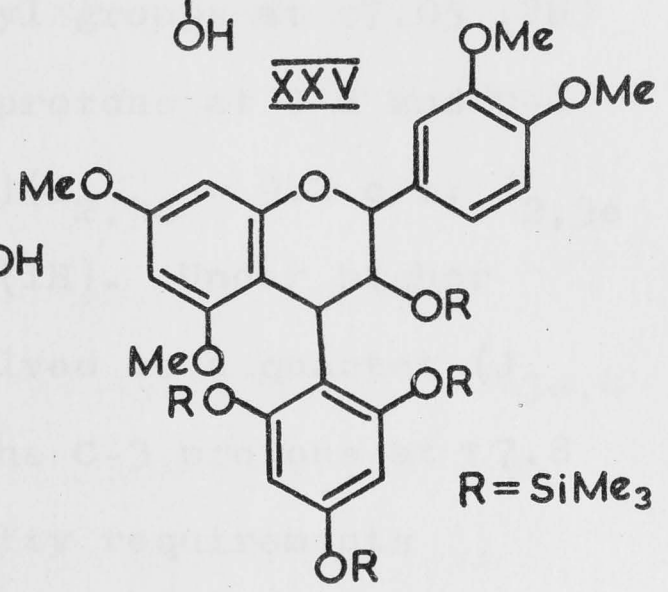
XXIV



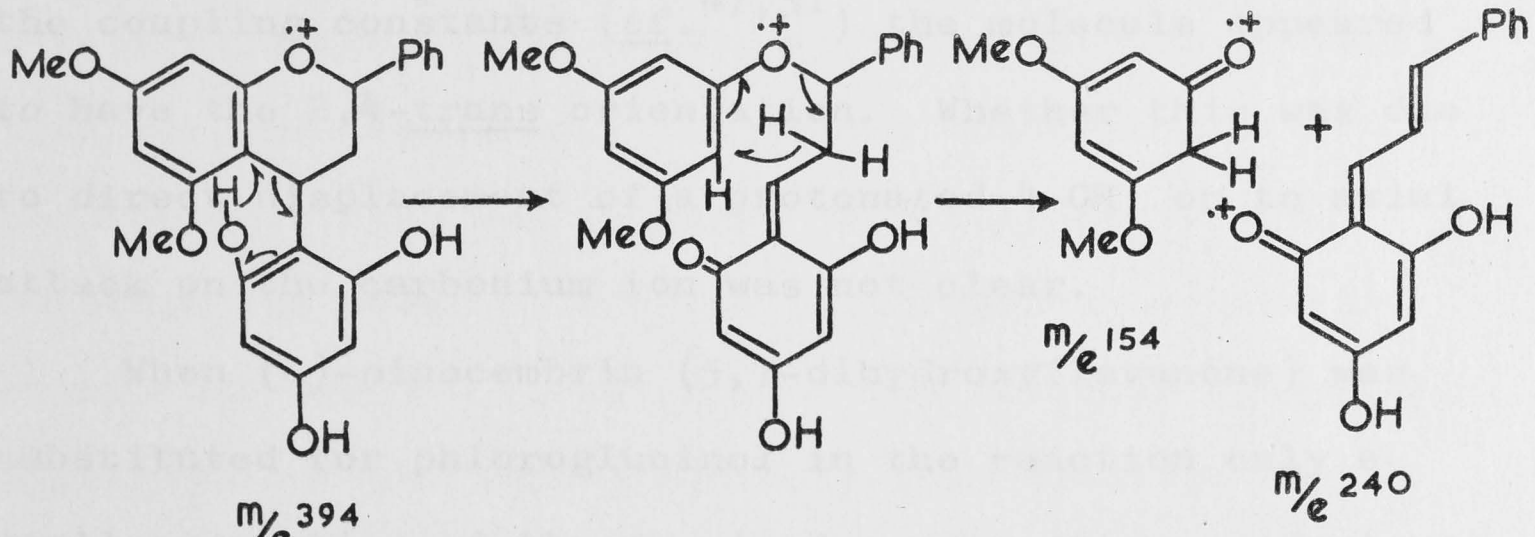
XXV



XXVI



XXVII



SCHEME 7

The proposed 2,4-*trans* structure for (XXV) was supported by its p.m.r. spectrum. Nine aromatic proton absorptions were observed, those of ring-A as a slightly distorted singlet at τ 3.88, those on ring-B as a multiplet centred at τ 2.65, and the two phoroglucinol protons as a sharp singlet τ 4.08. The methoxyl groups appeared at τ 6.26 and τ 6.38, and the hydroxyl groups at τ 7.05 (2H) and τ 2.18 (1H). The aliphatic protons at C-2 and C-4 produced a quartet at τ 4.72 (1H)($J_{2,3a}$ = 9.2 c/s; $J_{2,3e}$ = 4.8 c/s) and a triplet τ 5.36 (1H). Under higher resolution the triplet was resolved as a quartet ($J_{3e,4}$ = 3.3 c/s; $J_{3a,4}$ = 4.0 c/s). The C-3 protons at τ 7.8 were partly obscured as solubility requirements necessitated the use of deuteroacetone as solvent.

It was clear from the spectra that phloroglucinol was attached to the 4-position through carbon, and from the coupling constants (*cf.*[47,51]) the molecule appeared to have the 2,4-*trans* orientation. Whether this was due to direct displacement of a protonated 4-OH, or to axial attack on the carbonium ion was not clear.

When (-)-pinocembrin (5,7-dihydroxyflavanone) was substituted for phloroglucinol in the reaction only a small proportion of the required condensation product was obtained; the main product being dimeric or polymeric

flavan. The lower reactivity of pinocembrin was attributed to the presence of the carbonyl group. Purification of the product gave a racemic crystalline compound indistinguishable by m.p., m.m.p., t.l.c. and u.v. spectrometry from natural xanthorrhone. The mass spectra were virtually identical both in the positions and intensities of the peaks and the molecular ion for the synthetic material occurred at m/e 524.1838 (Calc. for $C_{32}H_{28}O_7$: 524.1835)(cf. Fig. 5).

There was insufficient material for a p.m.r. analysis so the only evidence available on the type of linkage in the synthetic material came from the mass spectral fragmentation pattern. In particular the relative heights of the peaks m/e 256 ($C_{15}H_{12}O_4$) to m/e 255 ($C_{15}H_{11}O_4$) (11% and 15%) and m/e 268($C_{17}H_{16}O_3$) to m/e 269 ($C_{17}H_{17}O_3$) (89% and 68%) indicated that direct cleavage of the units, with and without hydrogen transfer, was occurring. Therefore a non-aromatic heterocyclic ring was involved in the linkage. In addition the peaks at m/e 154 (phloroglucinol dimethyl ether) and m/e 370 (remainder of the molecule) suggested strongly that the diphenylmethane-type cleavage was again operating.

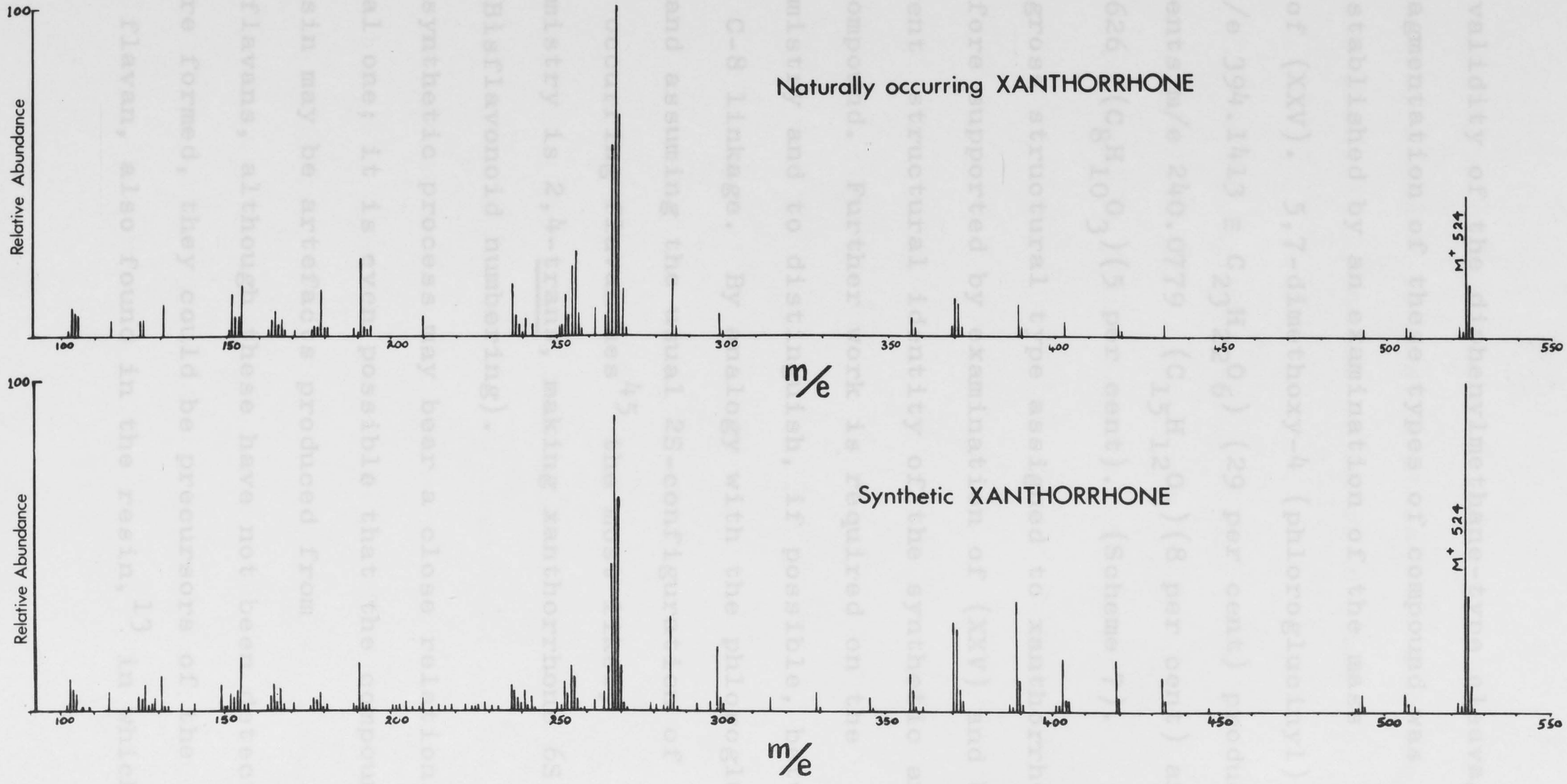

Naturally occurring XANTHORRHONE
Relative Abundance
100
150
200
250
300
350
400
450
500
550
m/e
M+ 524
Synthetic XANTHORRHONE
Relative Abundance
100
150
200
250
300
350
400
450
500
550
m/e
M+ 524

FIGURE 5

The validity of the diphenylmethane-type cleavage in the fragmentation of these types of compound was clearly established by an examination of the mass spectrum of (XXV). 5,7-dimethoxy-4 (phloroglucinyl) flavan (m/e 394.1413 ≡ $C_{23}H_{22}O_6$) (29 per cent) produced the fragments m/e 240.0779 ($C_{15}H_{12}O_3$)(8 per cent) and m/e 154.0626 ($C_8H_{10}O_3$)(5 per cent). (Scheme 7).

The gross structural type assigned to xanthorrhone was therefore supported by examination of (XXV) and by the apparent structural identity of the synthetic and natural compound. Further work is required on the stereochemistry and to distinguish, if possible, between a C-6 and C-8 linkage. By analogy with the phloroglucinol coupling and assuming the usual 2S-configuration of naturally occurring flavanones[45] the most likely stereochemistry is 2,4-trans, making xanthorrhone 6S, 8S, 24S (Bisflavonoid numbering).

The synthetic process may bear a close relation to the natural one; it is even possible that the compounds in the resin may be artefacts produced from 4-hydroxyflavans, although these have not been detected. If they are formed, they could be precursors of the monomeric flavan, also found in the resin,[13] in which

reductive removal of the OH occurs by a process mechanistically similar to that postulated[52] for the biosynthetic reductive removal of OH from cinnamyl alcohols.

PART III

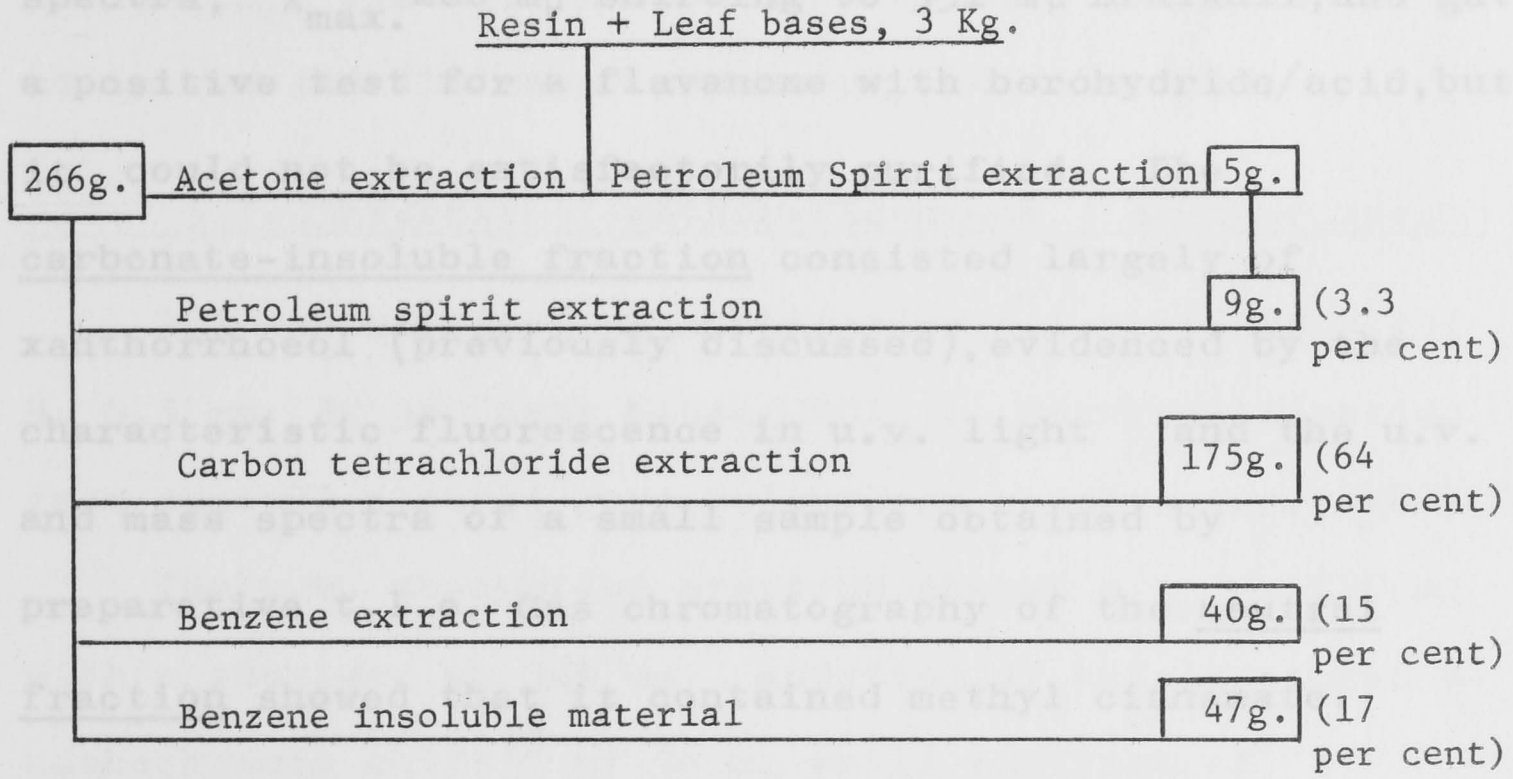
EXAMINATION OF THE RESIN FROM XANTHORRHOEA AUSTRALIS

A sample of Xanthorrhoea australis (including caudex, leaves, scape and spike) was selected from a colony of the species located at Mt. McDonald in the A.C.T. All the lower parts of the caudex which showed signs of charring from bushfires were discarded. The plant was then divided into three parts; (a) caudex containing brittle red resin, (b) leaves and leaf bases with adhering freshly exuded yellow sticky resin, (c) inflorescences (scape and spike).

(a) Brittle Resin

The trunk was stripped of excessive dry leaf, broken apart and the leaf bases with adhering resin placed in a large earthenware vessel. The material was then extracted, at room temperature, with several portions of petroleum spirit (b.p. 60^{o} - 80^{o}), then with a large volume of acetone. The acetone extract was concentrated to a black viscous tar which was then extracted with petroleum spirit (b.p. 60^{o} - 80^{o}), carbon tetrachloride

and benzene. The distribution of the resin was as follows:



Each of these extracts was divided up into bicarbonate-soluble, carbonate-soluble, and carbonate-insoluble fractions and examined by thin-layer chromatography. In all cases the bicarbonate-soluble fraction was very small and the ratio of the carbonate-soluble to carbonate-insoluble fractions was approximately 1 : 2 by weight.

Petrol Extract:

This fraction consisted largely of fat material (ca. 50 per cent) which was removed by precipitation from a methanolic solution. The remaining material appeared

on t.l.c. as a complex mixture. The carbonate-soluble fraction exhibited flavonoid-type u.v. absorption spectra, $\lambda_{max.}$ 288 mμ shifting to 331 mμ in alkali*, and gave a positive test for a flavanone with borohydride/acid, but it could not be satisfactorily purified. The carbonate-insoluble fraction consisted largely of xanthorrhoeol (previously discussed), evidenced by the characteristic fluorescence in u.v. light and the u.v. and mass spectra of a small sample obtained by preparative t.l.c. Gas chromatography of the neutral fraction showed that it contained methyl cinnamate.

Carbon Tetrachloride Extract:

The bicarbonate-soluble fraction was found to consist almost entirely of cinnamic and coumaric acids (ca. 1:4). (t.l.c., u.v.).

The u.v. absorption spectra of the carbonate-soluble fraction showed that the major chromophores in the mixture belonged to flavanones ($\lambda_{max.}$ 288 mμ ; $\Delta\lambda_{max.}$ OH^- = +47 mμ). This sticky brown material gave a positive Shinoda test[36] and a magenta colour with borohydride/acid. A sample of this mixture was taken up in benzene and passed down a silica column with chloroform as eluant. The eluted solution was evaporated under reduced pressure

* i.e. 2N NaOH

and the amorphous material obtained was purified by preparative t.l.c. (Silica gel (H), 0.5 mm; $CHCl_3$, plate run 3 times). A band at Rf = 0.40 (P22) was collected and its major component characterised.

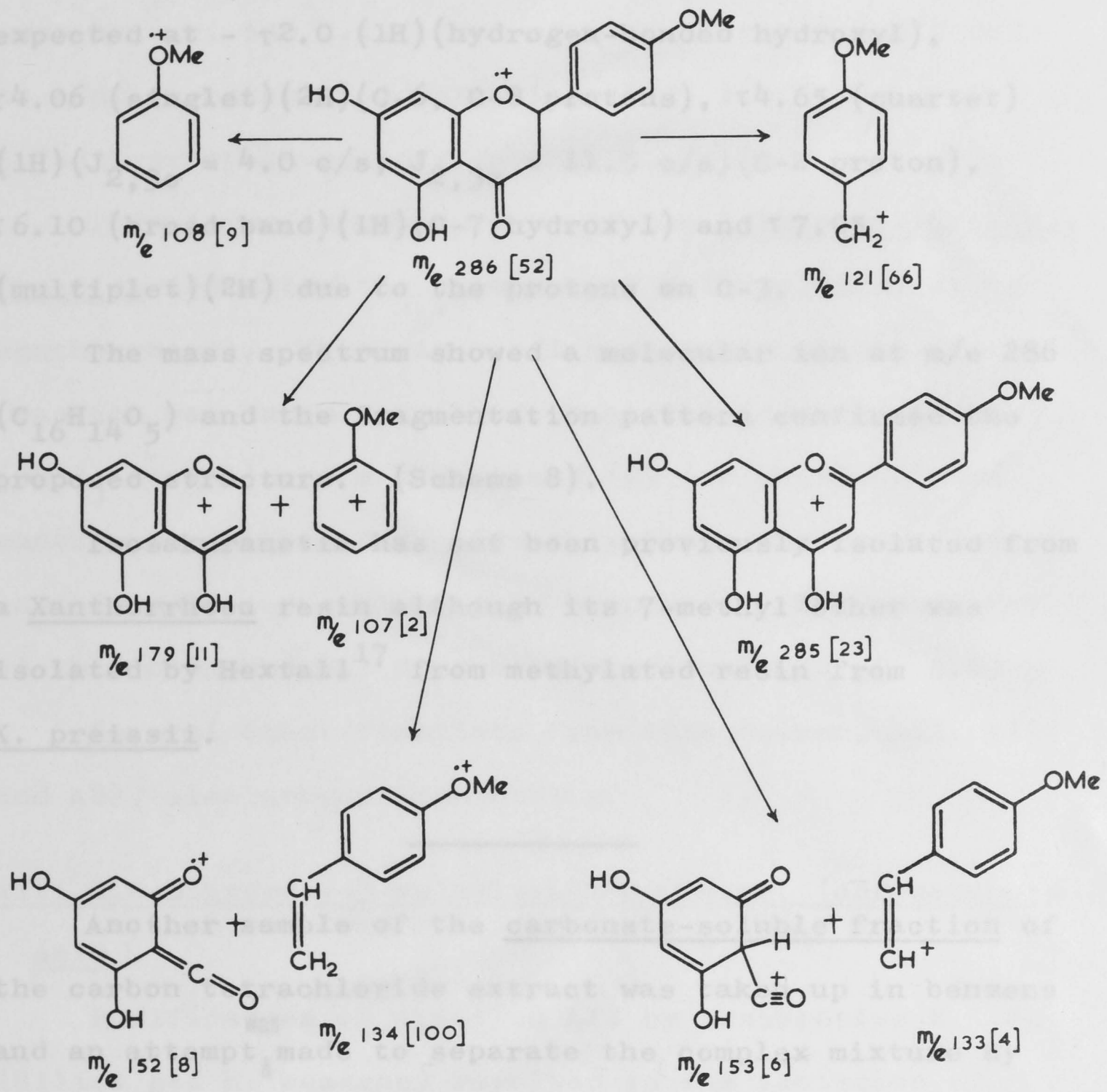
P22: 5,7-Dihydroxy-4'-methoxyflavanone. [Isosakuranetin]

P22 was purified by preparative t.l.c. (Silica gel H, 0.5 mm; 20 per cent EtOAc/$CHCl_3$). Recrystallisation from aqueous alcohol gave colourless needles m.p. 188-190°.

The u.v. absorption spectrum showed a $\lambda_{max.}$ 290 mμ with a shoulder at 329 mμ. Addition of alkali* caused a bathochromic shift[33] of 38 mμ while addition of aluminium chloride[34] produced a shift of 19 mμ. Sodium acetate[35] caused the long wavelength shoulder to be resolved. Therefore the C-5 and C-7 hydroxyl groups of the flavanone were unmethylated. The substance gave a positive Shinoda test[36] and a magenta colour with borohydride/acid.

A 100 Mc./sec. p.m.r. spectrum was in agreement with that expected for 5,7-dihydroxy-4'-methoxyflavanone. In particular, methoxyl proton absorption occurred at τ6.22 (3H) and the B-ring aromatic protons produced an A_2B_2 system appearing as doublets at τ 2.68 (2H)(C-2', C-6' protons) and τ 3.10 (2H)(C-3', C-5' protons),

* i.e. 2N NaOH


OMe
HO
O
OH
O
m/e 108 [9]
m/e 286 [52]
m/e 121 [66]
CH2
m/e 179 [11]
m/e 107 [2]
m/e 285 [23]
m/e 152 [8]
m/e 134 [100]
m/e 153 [6]
m/e 133 [4]
-CO
m/e 124 [5]

SCHEME 8

thereby establishing the substitution pattern of this ring. The remaining proton absorptions appeared as expected at - τ2.0 (1H)(hydrogen-bonded hydroxyl), τ4.06 (singlet)(2H)(C-6, C-8 protons), τ4.65 (quartet) (1H)($J_{2,3e}$ = 4.0 c/s, $J_{2,3a}$ = 11.5 c/s)(C-2 proton), τ6.10 (broad band)(1H)(C-7 hydroxyl) and τ7.05 (multiplet)(2H) due to the protons on C-3.

The mass spectrum showed a molecular ion at m/e 286 ($C_{16}H_{14}O_5$) and the fragmentation pattern confirmed the proposed structure. (Scheme 8).

Isosakuranetin has not been previously isolated from a Xanthorrhoea resin although its 7-methyl ether was isolated by Hextall[17] from methylated resin from X. preissii.

Another sample of the carbonate-soluble fraction of the carbon tetrachloride extract was taken up in benzene and an attempt was made to separate the complex mixture by counter current distribution against an aqueous buffer (pH 9). Unfortunately a combination of surface and solubility effects prevented the achievement of worthwhile separations. (This was in line with the results obtained by another worker[53] who examined

Xanthorrhoea resin by this technique). Nevertheless, a sample from the upper phase which appeared reasonably pure by u.v. and t.l.c. was examined further and found to contain material identical to P22, i.e. 5,7-dihydroxy-4'-methoxyflavanone.

Thin-layer chromatography of the carbonate-insoluble fraction indicated the presence of a large quantity of xanthorrhoeol. This was confirmed when a sample of this fraction was chromatographed on a silicic acid column ($CHCl_3$ eluant) and a high yield (29 per cent) of pure xanthorrhoeol obtained. (On this basis the crude resin contained 12 per cent of this compound). The chemistry of this compound was discussed previously. (cf. p.27)

Several other fractions from this column, A12, A52 and A81, also proved interesting.

A12: 1,8-Dihydroxy-3-methyl-anthraquinone. [chrysophanic acid]

Purification of fraction A12 by preparative t.l.c. (Silica gel H; benzene) resulted in the isolation of material, deep yellow in colour, with physical and chemical properties quite distinct from previously isolated compounds. It gave negative tests when treated with Mg/HCl or $NaBH_4$ followed by acid, and gave a violet-red colouration when treated with alkali.

Further purification by recrystallisation from aqueous alcohol or by sublimation gave yellow plates, m.p. 195-196°.

The u.v. spectrum (EtOH) exhibited $\lambda_{max.}$ at 225.5 mμ, 256.5 mμ, 278 mμ, 288 mμ, 320 mμ (sh.) and 430 mμ. On the addition of alkali* $\lambda_{max.}$ appeared at 210 mμ, 235 mμ, 285 mμ and 514 mμ. (cf. Fig. 6). The spectrum agrees with that given by Scott[54] for "chrysophanol" (≡ chrysophanic acid) and distinguishes it from 9 other hydroxyanthraquinones listed. The i.r. absorption spectrum (nujol) was in general agreement with this assignment and showed two carbonyl absorption bands; one at 1680 cm^{-1} (medium) and the other at 1630 cm^{-1} (strong).

The mass spectrum of the compound showed a molecular ion and base peak at m/e 254 ($C_{15}H_{10}O_4$). No other peak (with the exception of P + 1 [17 per cent]) exceeded 10 per cent relative abundance; those that were observed arose mainly from losses of 28 (CO). (cf. Scheme 9). An unusual feature in the spectrum of unsubstituted anthraquinone is the presence of a metastable peak corresponding to the transformation of one doubly-charged ion into another.[55] $(P\text{-}CO)^{2+} \longrightarrow (P\text{-}C_2O_2)^{2+} + CO$. Although a peak at m/e 127 was observed which might have corresponded to the doubly-charged parent, no such

* i.e. 2N NaOH

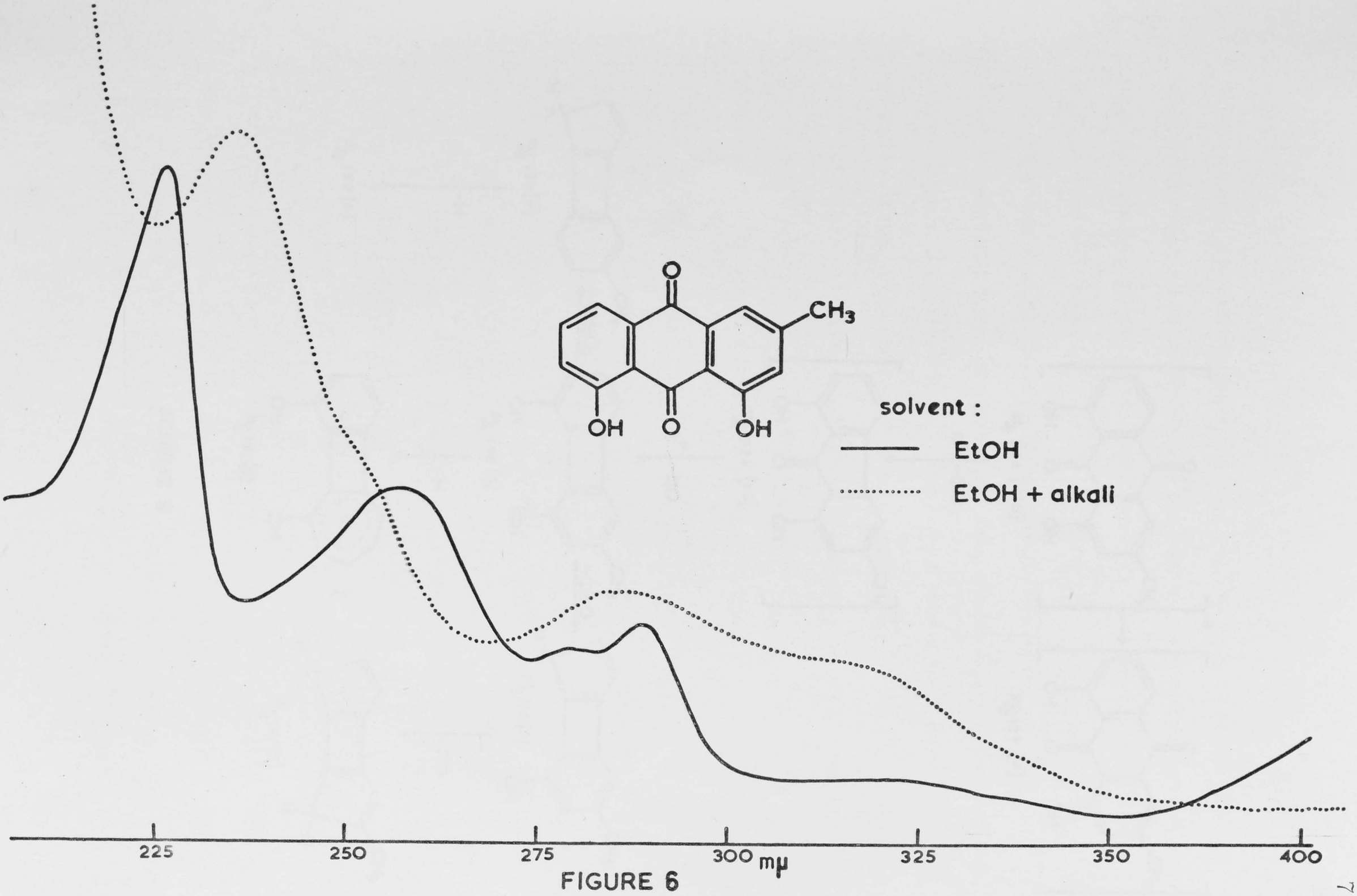
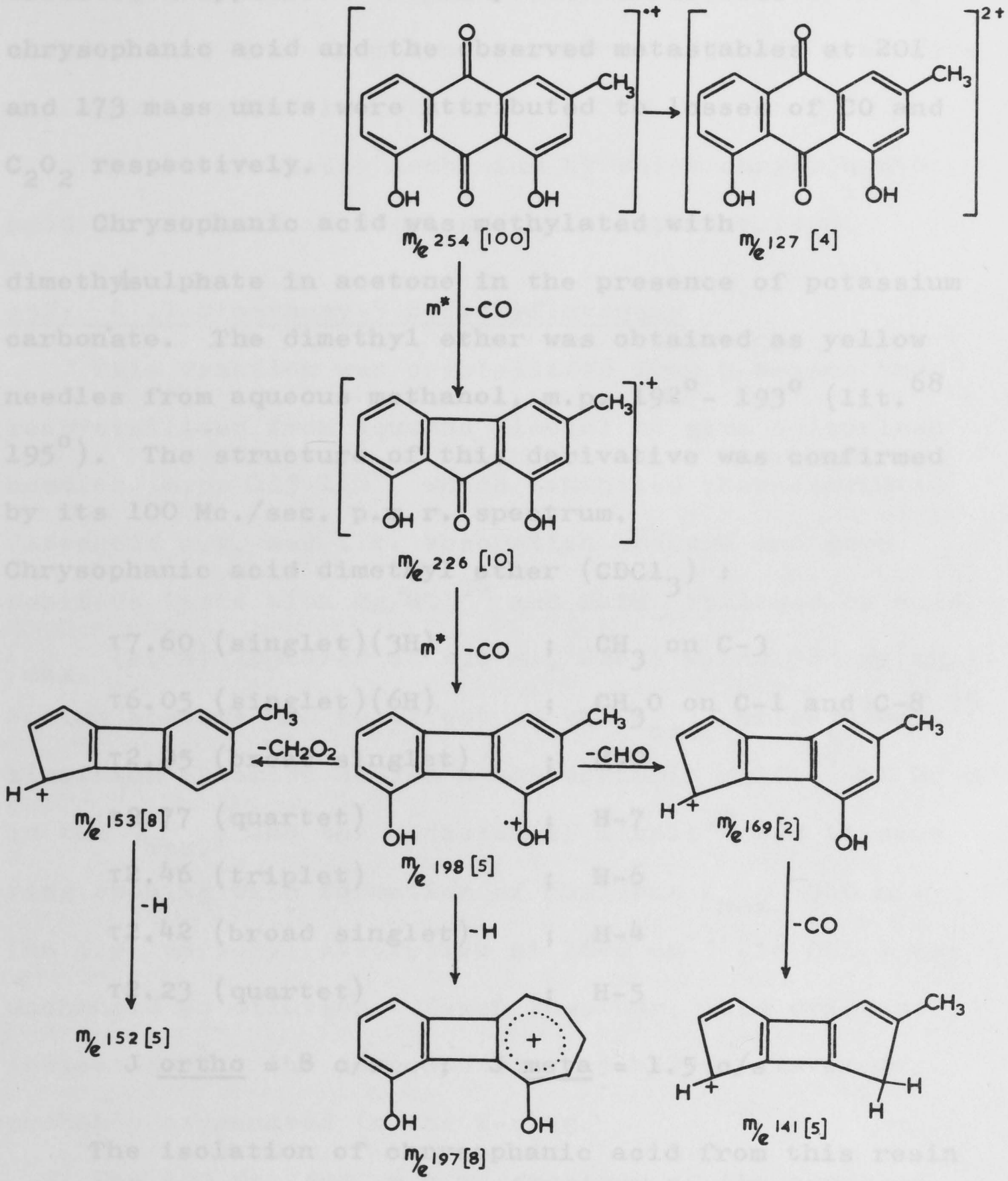

O
CH3
OH
O
OH
solvent :
EtOH
EtOH + alkali
225
250
275
300 mμ
325
350
400

FIGURE 6


m/e 254 [100]
m/e 127 [4]
m* -CO
m/e 226 [10]
m* -CO
-CH2O2
-CHO
m/e 153 [8]
m/e 198 [5]
m/e 169 [2]
-H
-H
-CO
m/e 152 [5]
m/e 197 [8]
m/e 141 [5]

SCHEME 9

transition appeared to take place in the case of chrysophanic acid and the observed metastables at 201 and 173 mass units were attributed to losses of CO and C_2O_2 respectively.

Chrysophanic acid was methylated with dimethylsulphate in acetone in the presence of potassium carbonate. The dimethyl ether was obtained as yellow needles from aqueous methanol, m.p. 192°- 193° (lit.[68] 195°). The structure of this derivative was confirmed by its 100 Mc./sec. p.m.r. spectrum.

Chrysophanic acid dimethyl ether ($CDCl_3$) :

τ7.60 (singlet)(3H)	;	CH_3 on C-3
τ6.05 (singlet)(6H)	;	CH_3O on C-1 and C-8
τ2.95 (broad singlet)	;	H-2
τ2.77 (quartet)	;	H-7
τ2.46 (triplet)	;	H-6
τ2.42 (broad singlet)	;	H-4
τ2.23 (quartet)	;	H-5

J ortho = 8 c/s ; J meta = 1.5 c/s

The isolation of chrysophanic acid from this resin was of considerable interest as it was previously thought to arise only as an artefact in material directly damaged by bushfires.[22] The plant material used in this

investigation showed no signs of charring so it was assumed that heat from sunlight or an adjacent grassfire was equally effective.

The biosynthetic mechanism by which chrysophanic acid is produced is discussed in a later section.

A52: 4',7-dimethoxy-5-hydroxyflavanone

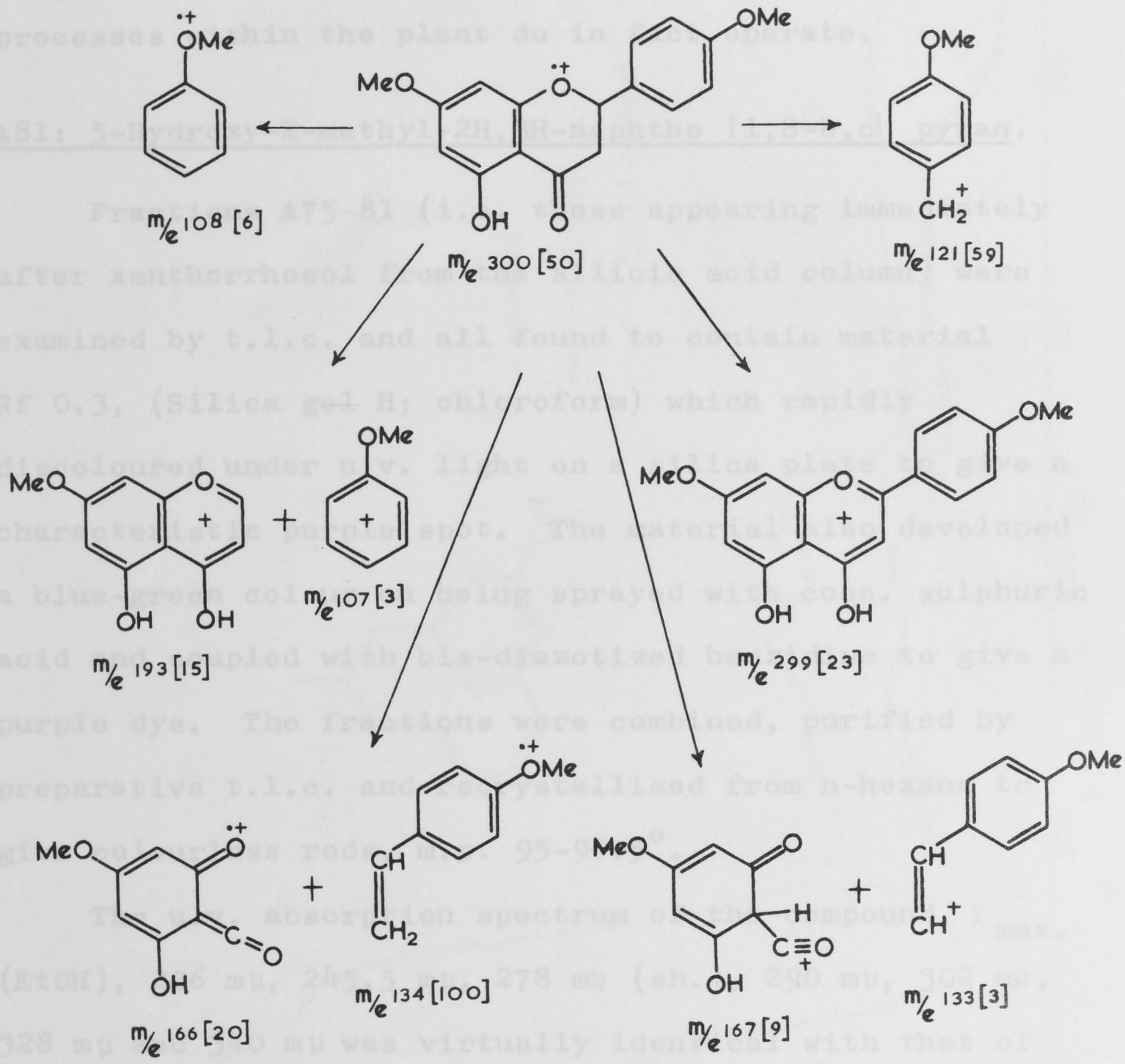
This fraction was crystallised from n-hexane then recrystallised from aqueous alcohol to give colourless needles, m.p. 115-116°, which exhibited characteristic flavonoid u.v. and i.r. absorption spectra and gave positive tests with Mg/HCl[36] and $NaBH_4$ followed by acid. $\lambda_{max.}$ (EtOH) appeared at 226 mμ, 288.5 mμ and 328 mμ (sh.). Sodium acetate had no effect on the $\lambda_{max.}$ or shoulder,[35] aluminium chloride caused a bathochromic shift[34] of 20 mμ in the $\lambda_{max.}$, and the addition of alkali[33] led to some ring opening with formation of chalcone $\lambda_{max.}$ 360 mμ. The i.r. carbonyl absorption at 1640 cm^{-1} (in CCl_4) was unchanged on dilution. Taken together, this evidence indicated that the compound was a 5-hydroxyflavanone, probably oxygenated in the B-ring.

The 100 Mc./sec. p.m.r. spectrum of the compound (in $CDCl_3$) confirmed this and provided some additional information. Of particular note were the two methoxyl

proton absorptions at τ6.19 and τ6.22, and the B-ring aromatic proton absorptions which appeared effectively as a four-peak pattern of an A_2B_2 system with doublets at τ2.63 (2H) and τ3.07 (2H). On this basis one methoxyl group could be assigned to the 4'-position of the B-ring and it seemed likely that the other was on C-7. The rest of the spectrum did, in fact, support this proposal and it was concluded that the compound was 4',7-dimethoxy-5-hydroxyflavanone. The hydrogen-bonded hydroxyl proton absorbed at - τ2.0, the protons on C-6 and C-8 produced a singlet at τ3.96 and the aliphatic proton absorptions appeared as a quartet at τ4.65 (1H)($J_{2,3a}$ = 12.0 c/s; $J_{2,3e}$ = 3.9 c/s) due to the proton on C-2 and a multiplet at τ7.0 (2H)($J_{3e,3a}$ = 17.0 c/s) due to the geminal protons on C-3.

A mass spectrum of the compound confirmed the proposed structure. (Scheme 10). The molecular ion appeared at m/e 300 ($C_{17}H_{16}O_5$) and the normal fragmentation pattern for flavanones[37] was observed. The extinction coefficient log ε = 4.28 ($\lambda_{max.}$ 289 mμ) is of the same order as for other flavanones. (e.g. naringenin[28] $\lambda_{max.}$ 290 mμ (log ε = 4.29).

4',7-dimethoxy-5-hydroxyflavanone has previously been isolated from the methylated resin of a xanthorrhoea.[17]


OMe
MeO
O
OH
m/e 108 [6]
m/e 300 [50]
m/e 121 [59]
m/e 193 [15]
m/e 107 [3]
m/e 299 [23]
m/e 166 [20]
m/e 134 [100]
m/e 167 [9]
m/e 133 [3]

SCHEME 10

Its isolation in this case from an unmethylated resin serves to remove any doubt that extensive methylation processes within the plant do in fact operate.

A81: 5-Hydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran.

Fractions A75-81 (i.e. those appearing immediately after xanthorrhoeol from the silicic acid column) were examined by t.l.c. and all found to contain material Rf 0.3, (Silica gel H; chloroform) which rapidly discoloured under u.v. light on a silica plate to give a characteristic purple spot. The material also developed a blue-green colour on being sprayed with conc. sulphuric acid and coupled with bis-diazotized benzidine to give a purple dye. The fractions were combined, purified by preparative t.l.c. and recrystallised from n-hexane to give colourless rods, m.p. 95-96.5°.

The u.v. absorption spectrum of the compound, $\lambda_{max.}$ (EtOH), 226 mμ, 245.5 mμ, 278 mμ (sh.), 290 mμ, 302 mμ, 328 mμ and 340 mμ was virtually identical with that of xanthorrhoein (III), but gave a shift with alkali to $\lambda_{max.}$ 245 mμ, 251 mμ, 260 mμ (sh.), 283 mμ, 295 mμ, 308 mμ and 352 mμ. (cf. Fig. 7).

The 100 Mc./sec. p.m.r. spectra of the two compounds (in $CDCl_3$) were also very similar. The spectra of the

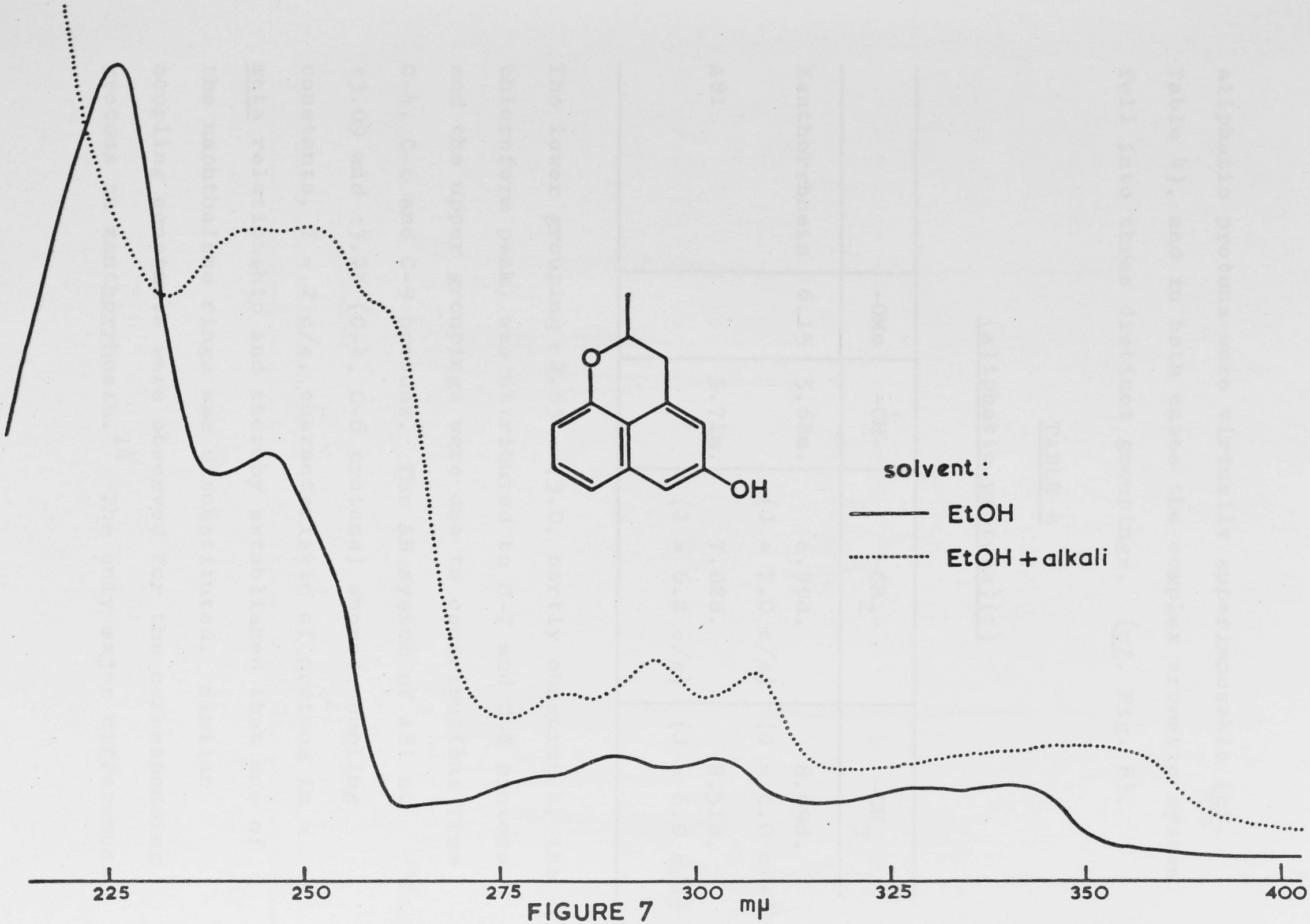


FIGURE 7

aliphatic protons were virtually superimposable (cf. Table 4), and in both cases the complex aromatic system fell into three distinct groupings. (cf. Fig. 8).

TABLE 4

(Aliphatic protons)(τ)

	-OMe	-CH-	$-CH_2-$	$-CH_3$
Xanthorrhoein	6.15	5.68m.	6.99d. (J = 7.0 c/s)	8.49d. (J = 6.0 c/s)
A81		5.71m.	7.02d. (J = 6.2 c/s)	8.51d. (J = 6.0 c/s)

The lower grouping τ 2.65 - τ3.0, partly obscured by the chloroform peak, was attributed to C-7 and C-8 protons and the upper groupings were due to contributions from C-4, C-6 and C-9 protons. The AB system of A81 at τ3.09 and τ3.25 (C-4, C-6 protons) showed coupling constants, J = 2 c/s, characteristic of protons in a meta relationship and thereby established that one of the naphthalene rings was disubstituted. Similar coupling constants were observed for the corresponding protons in xanthorrhoein.[14] The only major differences

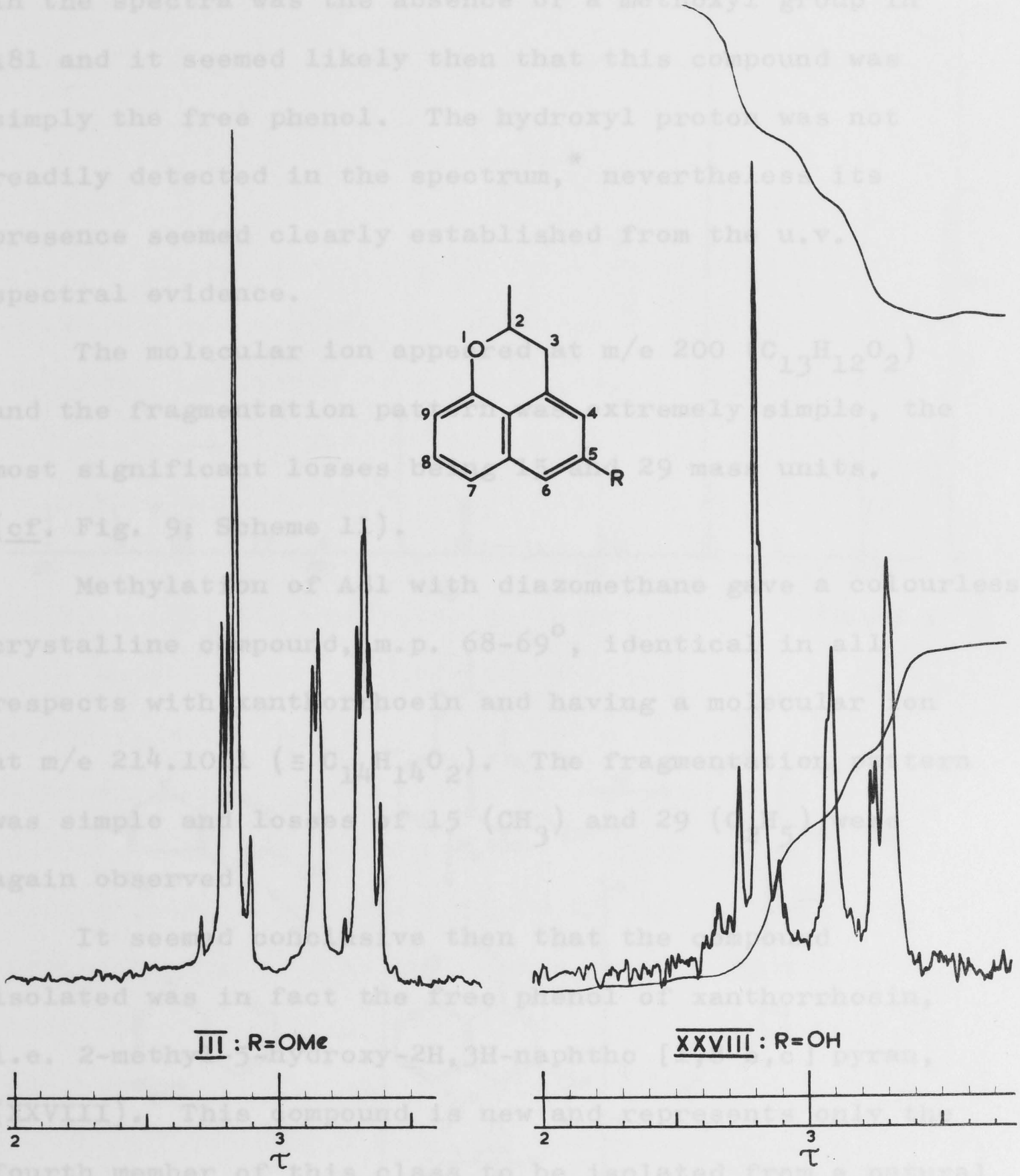


FIGURE 8

in the spectra was the absence of a methoxyl group in A81 and it seemed likely then that this compound was simply the free phenol. The hydroxyl proton was not readily detected in the spectrum,* nevertheless its presence seemed clearly established from the u.v. spectral evidence.

The molecular ion appeared at m/e 200 ($C_{13}H_{12}O_2$) and the fragmentation pattern was extremely simple, the most significant losses being 15 and 29 mass units. (cf. Fig. 9; Scheme 11).

Methylation of A81 with diazomethane gave a colourless crystalline compound, m.p. 68-69°, identical in all respects with xanthorrhoein and having a molecular ion at m/e 214.1001 ($\equiv C_{14}H_{14}O_2$). The fragmentation pattern was simple and losses of 15 (CH_3) and 29 (C_2H_5) were again observed.

It seemed conclusive then that the compound isolated was in fact the free phenol of xanthorrhoein, i.e. 2-methyl-5-hydroxy-2H,3H-naphtho [1,8-b,c] pyran, (XXVIII). This compound is new and represents only the fourth member of this class to be isolated from a natural

---

*

However, the multiplet at τ5.7 (integrating for slightly more than one proton) had a broad base which was removed by the addition of D_2O.

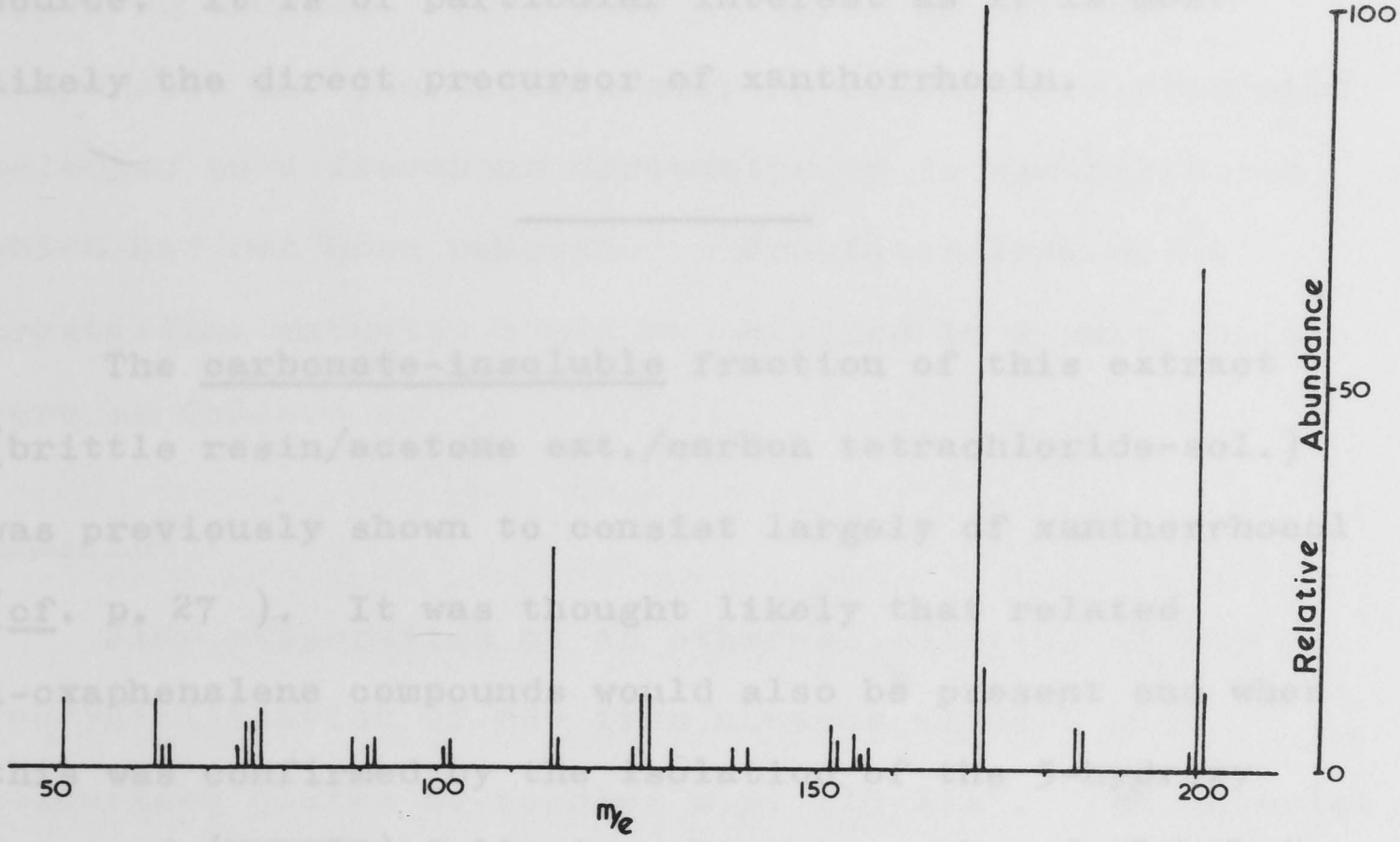
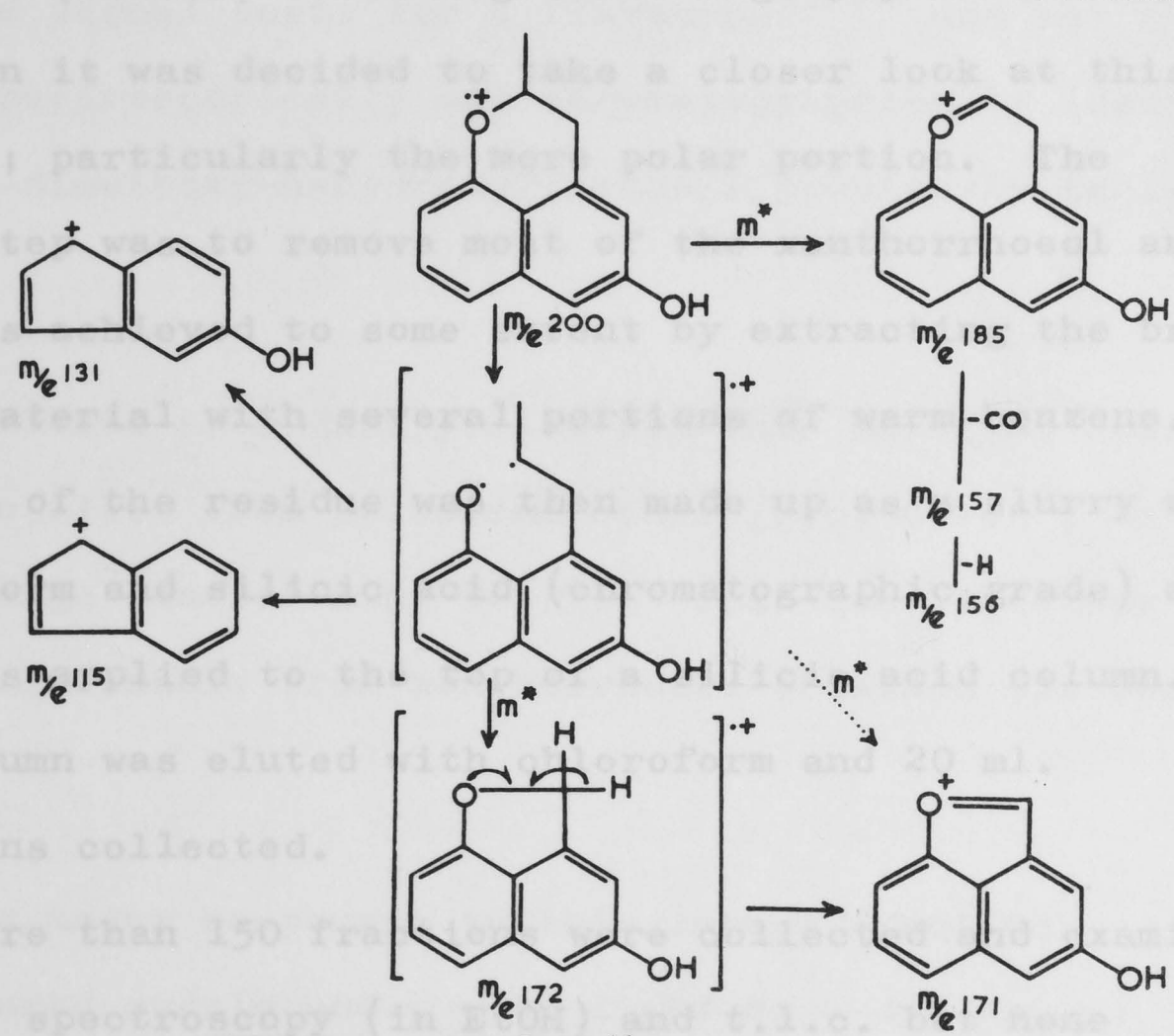


FIGURE 9



SCHEME 11

source. It is of particular interest as it is most likely the direct precursor of xanthorrhoein.

The carbonate-insoluble fraction of this extract (brittle resin/acetone ext./carbon tetrachloride-sol.) was previously shown to consist largely of xanthorrhoeol (cf. p. 27). It was thought likely that related 1-oxaphenalene compounds would also be present and when this was confirmed by the isolation of the 5-hydroxy compound (XXVIII) following chromatography of the whole fraction it was decided to take a closer look at this extract; particularly the more polar portion. The first step was to remove most of the xanthorrhoeol and this was achieved to some extent by extracting the brown tarry material with several portions of warm benzene. Portion of the residue was then made up as a slurry with chloroform and silicic acid (chromatographic grade) and this was applied to the top of a silicic acid column. The column was eluted with chloroform and 20 ml. fractions collected.

More than 150 fractions were collected and examined by u.v. spectroscopy (in EtOH) and t.l.c. but none

appeared unusual. Most were mixtures of several components and the major absorbing chromophore frequently belonged to a flavanone derivative or to xanthorrhoeol which had not been removed. Fractions from which crystalline material could be obtained in a pure state were as follows :-

C19, C24 :

Slow evaporation of an ethereal solution of C19 or recrystallisation of C24 from aqueous alcohol gave colourless plates or needles m.p. 115-116°. The material gave the normal tests for a flavanone[33-36] and was shown to be spectroscopically and chromatographically identical with 4',7-dimethoxy-5-hydroxyflavanone previously isolated.

C50 :

Obtained as a dark yellow oil this material could be recrystallised from petroleum ether (b.p. 60-80°) to give orange "warts" m.p. 96-97°. This substance was indistinguishable from xanthorrhoeol obtained elsewhere.

C98 :

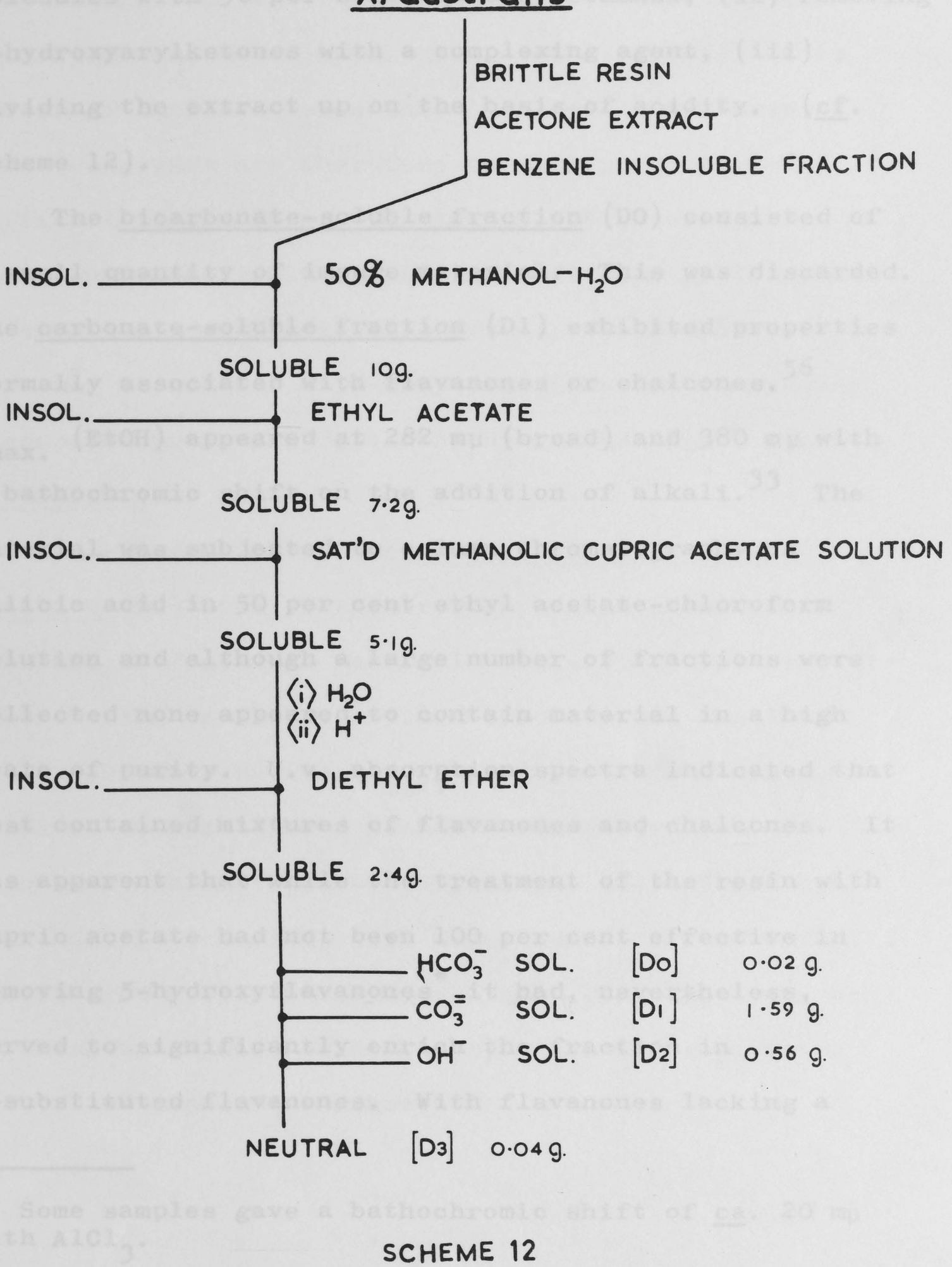
The major component in this impure fraction discoloured purple under u.v. light on a silica gel thin-layer plate. Purification of the fraction by

preparative t.l.c. on silica gel (H) resulted in the isolation of material spectroscopically identical to 5-hydroxy-2-methyl-2H, 3H-naphtho [1,8 - b,c] pyran. This was methylated with diazomethane and passed down a neutral alumina column in ether. A colourless crystalline material m.p. 63-65° (M^+ 214) identical to xanthorrhoein (III) was obtained.

Many of the miscellaneous slightly impure fractions exhibited u.v. absorption spectra normally characteristic of flavanones. Examination of these by mass spectroscopy failed to produce any evidence to suggest that dimeric flavonoids were present. Most appeared to be mixtures of naringenin methyl ethers.

Benzene-Insoluble Extractive

Direct chromatography of the previous fractions (CCl_4 extract/carbonate-insoluble/benzene-insoluble) had failed to yield any new compounds and it was thought that a similar procedure with this extract would be unjustified. A new approach to the problem was developed therefore which would afford a greater degree of preliminary separation of the resin prior to chromatography. The scheme devised aimed at (i) separating the polymeric material from the "smaller"


X. australis
BRITTLE RESIN
ACETONE EXTRACT
BENZENE INSOLUBLE FRACTION
INSOL.
50% METHANOL–H2O
SOLUBLE 10g.
INSOL.
ETHYL ACETATE
SOLUBLE 7·2g.
INSOL.
SAT'D METHANOLIC CUPRIC ACETATE SOLUTION
SOLUBLE 5·1g.
⟨i⟩ H2O
⟨ii⟩ H+
INSOL.
DIETHYL ETHER
SOLUBLE 2·4g.
HCO3− SOL. [Do] 0·02 g.
CO3= SOL. [D1] 1·59 g.
OH− SOL. [D2] 0·56 g.
NEUTRAL [D3] 0·04 g.

SCHEME 12

molecules with 50 per cent aqueous methanol, (ii) removing o-hydroxyarylketones with a complexing agent, (iii) dividing the extract up on the basis of acidity. (cf. Scheme 12).

The bicarbonate-soluble fraction (D0) consisted of a small quantity of impure material. This was discarded. The carbonate-soluble fraction (D1) exhibited properties normally associated with flavanones or chalcones.[56] $\lambda_{max.}$ (EtOH) appeared at 282 mμ (broad) and 380 mμ with a bathochromic shift on the addition of alkali.[33] The material was subjected to column chromatography on silicic acid in 50 per cent ethyl acetate-chloroform solution and although a large number of fractions were collected none appeared to contain material in a high state of purity. U.v. absorption spectra indicated that most contained mixtures of flavanones and chalcones. It was apparent that while the treatment of the resin with cupric acetate had not been 100 per cent effective in removing 5-hydroxyflavanones* it had, nevertheless, served to significantly enrich the fraction in 5-substituted flavanones. With flavanones lacking a

---

* Some samples gave a bathochromic shift of ca. 20 mμ with $AlCl_3$.

5-hydroxyl group, the equilibrium of the chalcone-flavanone isomerisation, under mildly basic conditions, lies in favour of chalcone formation.[57] Isomeric mixtures of these compounds are therefore readily produced and difficult to separate. In the present investigation only one column fraction was purified sufficiently to permit a tentative identification. From spectroscopic data it appeared to contain 4',7-dihydroxy-5-methoxyflavanone; a compound previously isolated by Salahud-Din[28] from X. preissii.

An examination of the sodium hydroxide-soluble fraction (D2) by u.v. spectroscopy again showed the presence of flavonoid material. This time, however, its contribution to the absorption spectrum was small and the major contribution ($\lambda_{max.}$ at 225 mμ and 246 mμ) appeared to come from a naphthopyran derivative. Thin-layer chromatography (Silica gel (H); 25 per cent EtOAc-$CHCl_3$) revealed five major bands, two of which discoloured purple under u.v. light. Samples from each of these bands were collected by means of preparative t.l.c. and examined further.

D27	Rf 0.58	yellow
D26	Rf 0.53	colourless (discoloured under u.v.)
D25	Rf 0.48	yellow
D23	Rf 0.27	yellow
D21	Rf 0.14	colourless (discoloured under u.v.)

D27 :

This material was identified as xanthorrhoeol.

D26 :

This substance was indistinguishable by normal physical and spectroscopic methods from 5-hydroxy-2-methyl-2H, 3H-naphtho [1,8 - b,c] pyran.

D25 :

The u.v. absorption spectrum indicated that the sample contained flavanone and chalcone material, while mass spectrometry revealed that at least 3 compounds were present. One of these appeared to be a methoxydihydroxychalcone of molecular weight 270, possibly 4'-methoxy-2', 4-dihydroxychalcone.

D23 : 4,4'-Dimethoxy-2',3,5,-trihydroxychalcone.

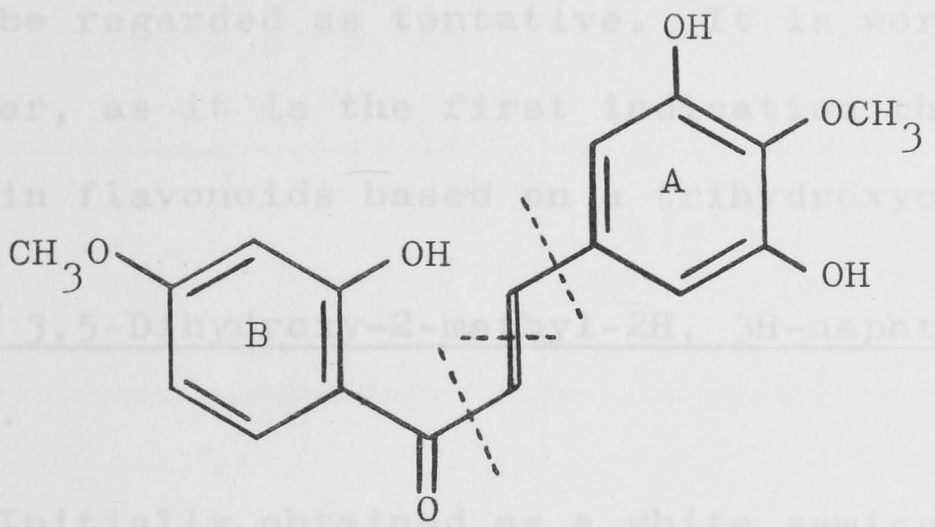
The fraction D23 was purified by preparative t.l.c. on silica gel and recrystallised from a benzene-chloroform

solution as yellow needles, m.p. 180-182°. The compound appeared to be a chalcone as it exhibited a u.v. absorption spectra, $\lambda_{max.}$ = 368 mμ, normally characteristic of this class of compound and produced an orange-red colouration on treatment with antimony pentachloride in carbon tetrachloride.[58]

As there was only a small amount of material available (ca. 7 mg.) structure elucidation relied on mass spectrometry. The molecular ion and base peak occurred at m/e 316.0902 ($C_{17}H_{16}O_6$) and significant fragments occurred at m/e 177.0550 ($C_{10}H_9O_3$)(35%), 166 (13%), 153.0552 ($C_8H_9O_3$)(40%) and 151.0390 ($C_8H_7O_3$) (84%).

Chalcones normally cleave at three positions: (i) the carbonyl-$^{\alpha}$C bond, (ii) the $^{\alpha}$C-$^{\beta}$C linkage and (iii) the $^{\beta}$C-Ring A bond. (Ring A in chalcones being ring B in the corresponding flavanones). Following cleavage (i) both fragments are usually observed, though the A-ring fragment frequently picks up a transferred hydrogen at $^{\alpha}$C. With cleavage (ii) only the A-ring fragment is observed and again a hydrogen transfer is involved, this time producing the tropylium ion or its equivalent. Cleavage (iii) is usually direct, the B-ring fragment being the more significant.

On the basis of mass measurements it was concluded that the substitution pattern of the chalcone D23 was such that the A-ring bore one methoxyl and two hydroxyl substituents and the B-ring one methoxyl and one hydroxyl substituent as in 4,4'-dimethoxy-2',3,5-trihydroxychalcone.



The particular substitution pattern for D23 suggested above is proposed mainly on the basis of biogenetic considerations and has not been fully substantiated. The B-ring methoxyl group is placed at C-4' rather than at C-6' only on the basis that 6'-deoxychalcones (and 5-deoxyflavanones) are more common in nature than 4'-deoxychalcones (and 7-deoxyflavanones)(cf.[59]). The u.v. spectra of phenolic compounds containing o-dihydroxyl groups are known to alter characteristically in the presence of a mixture of boric acid and sodium

acetate.[60] In the case of D23 this shift was not observed and the A-ring methoxyl group has therefore been placed at C-4.

4,4'-Dimethoxy-2',3,5-trihydroxychalcone does not appear to be mentioned in the literature, and in the absence of further evidence the proposed structure can only be regarded as tentative. It is worthy of mention, however, as it is the first indication that red resins contain flavonoids based on a trihydroxycinnamyl unit.

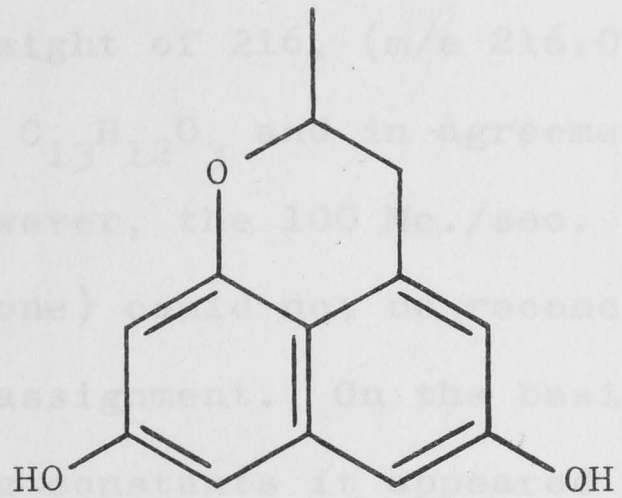
D21 : 3,5-Dihydroxy-2-methyl-2H, 3H-naphtho [1,8-b,c] pyran.

Initially obtained as a white semicrystalline solid m.p. 195-208° this material was recrystallised from benzene as colourless needles m.p. 211-214° (35 mg.). The u.v. absorption spectra of D21 in both neutral and basic solutions bore a close relationship to those of 5-hydroxy-2-methyl-2H, 3H-naphtho [1,8 - b,c] pyran. (cf. Table 5).

TABLE 5

Compound	$\lambda_{max.}$ (EtOH)
5-hydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran	226 mμ, 245.5 mμ, 278 mμ (sh.), 290 mμ, 302 mμ, 328 mμ, 340 mμ
D21	225.5 mμ, 245.5 mμ, 277 mμ (sh.), 289 mμ, 301.5 mμ, 327 mμ, 339 mμ

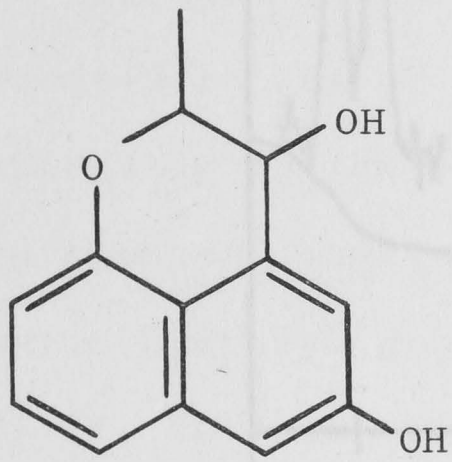
Compound	$\lambda_{max.}$ (EtOH/NaOH)
5-hydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran	245 mμ, 251 mμ, 260 mμ (sh.), 283 mμ, 295 mμ, 308 mμ, 352 mμ
D21	238.5 mμ, 251.5 mμ, 260 mμ (sh.), 283 mμ, 294 mμ, 306.5 mμ, 352 mμ



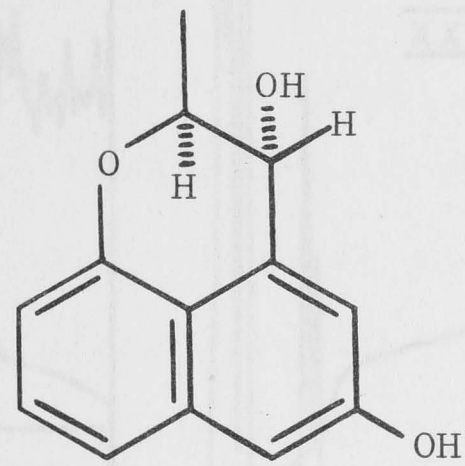
XXIX

The similarity in the spectra was a little surprising as a dihydroxynaphthopyran such as (XXIX), which was tentatively proposed as the structure on the basis of Rf values and biogenetic considerations, would be expected to show a more significant absorption shift on the addition of alkali. The i.r. absorption spectrum (nujol mull) showed strong absorption peaks at 3315 cm^{-1} and 3250 cm^{-1} due to hydroxyl absorption, a weak absorption peak at 3050 cm^{-1} (aromatic CH stretching[61]) and absorption maxima arising from aromatic C = C stretching at 1630 cm^{-1} (weak), 1610 cm^{-1} (medium) and 1595 cm^{-1} (medium). Further aromatic absorptions occurred at 1510 cm^{-1}, 830 cm^{-1}, 763 cm^{-1}, 773 cm^{-1} (all medium) and 855 cm^{-1} (strong). No carbonyl absorption was observed.

A mass measurement of purified D21 indicated a molecular weight of 216, (m/e 216.0783), equivalent to the formula $C_{13}H_{12}O_3$ and in agreement with structure (XXIX). However, the 100 Mc./sec. p.m.r. spectrum (in deuteroacetone) could not be reconciled with this structural assignment. On the basis of chemical shifts and coupling constants it appeared that the second hydroxyl group had been incorrectly located. Consistency with the observed spectrum was obtained by placing the hydroxyl group on C-3 as in (XXX).

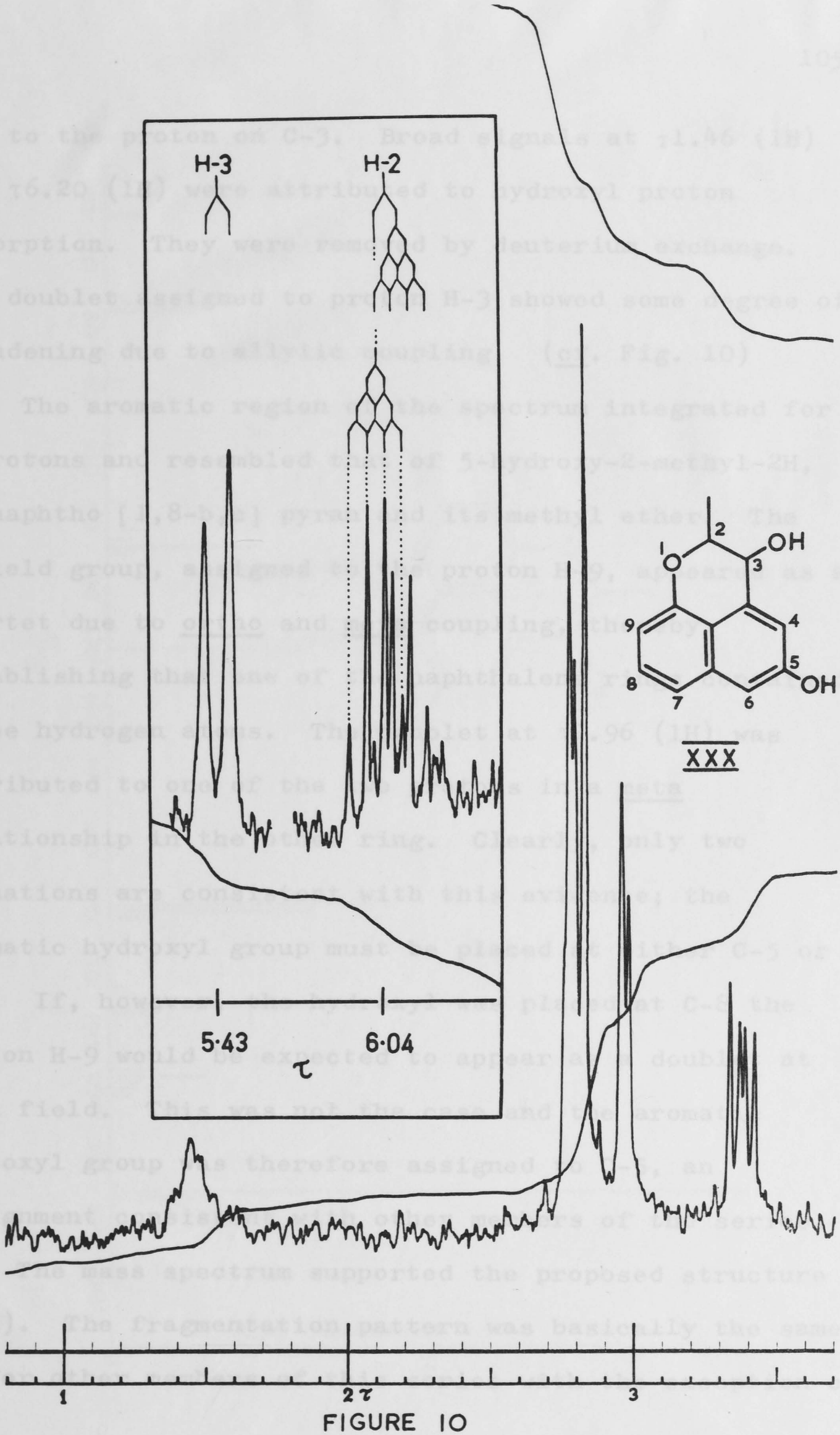


XXX



XXXI

The spectrum showed aliphatic proton absorption at τ8.49 (doublet)(3H)(J = 6.0 c/s) due to the methyl group, a multiplet (2 overlapping quartets) at τ6.04 (1H) due to the C-2 proton and a doublet at τ 5.43 (1H)(J = 8.5 c/s)



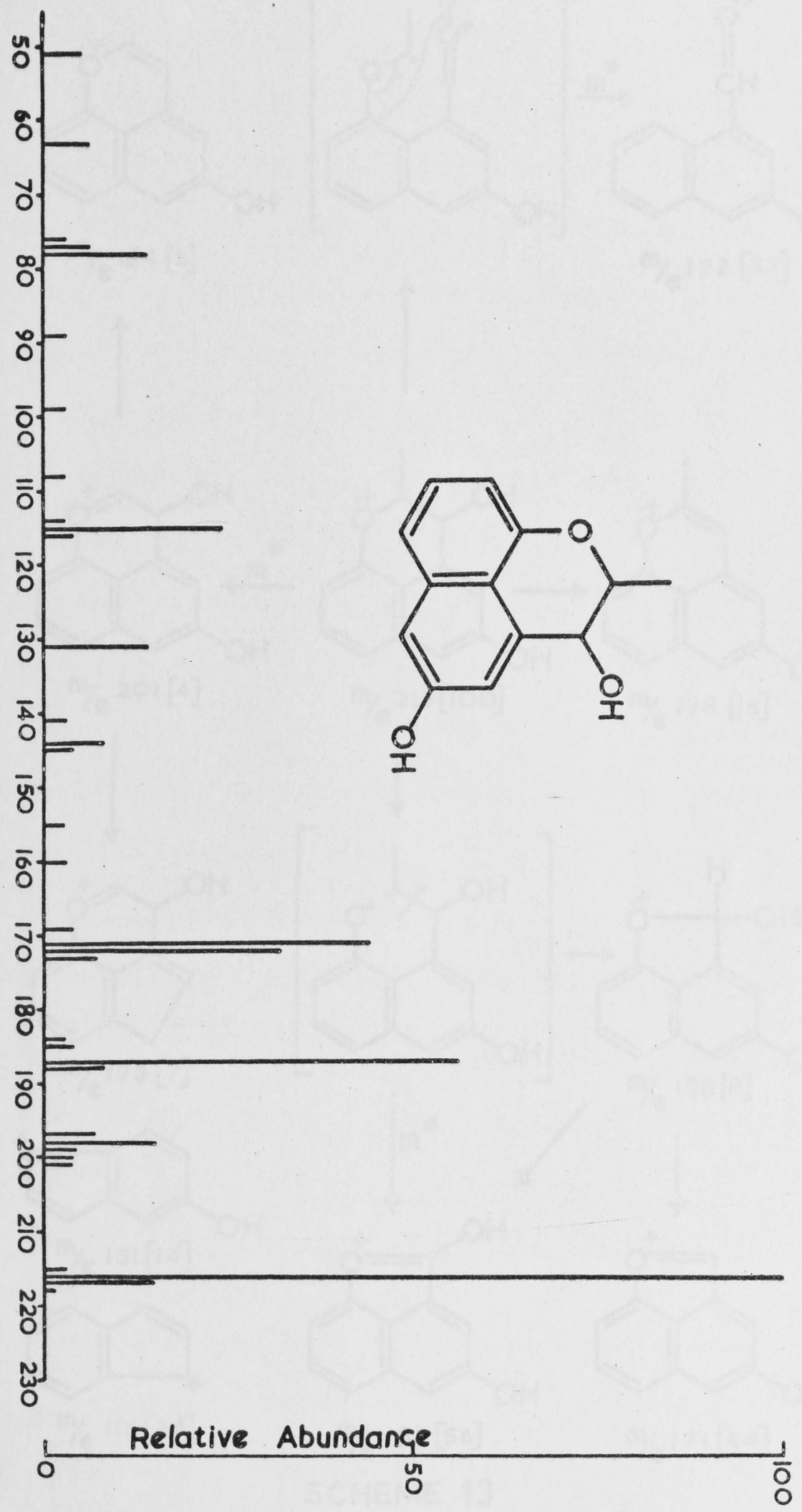
XXX

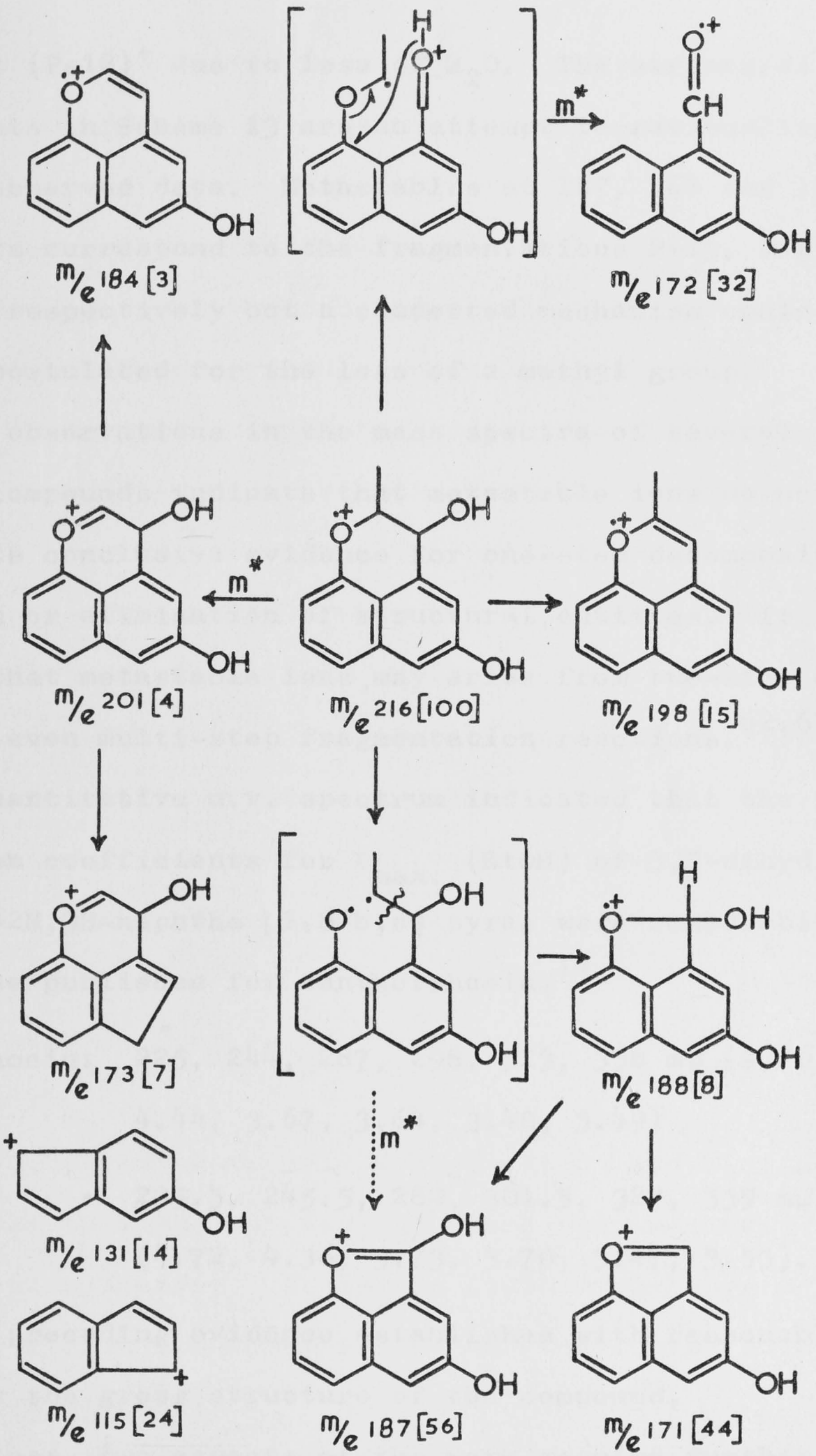
FIGURE 10

due to the proton on C-3. Broad signals at τ1.46 (1H) and τ6.20 (1H) were attributed to hydroxyl proton absorption. They were removed by deuterium exchange. The doublet assigned to proton H-3 showed some degree of broadening due to allylic coupling. (cf. Fig. 10)

The aromatic region of the spectrum integrated for 5 protons and resembled that of 5-hydroxy-2-methyl-2H, 3H-naphtho [1,8-b,c] pyran and its methyl ether. The upfield group, assigned to the proton H-9, appeared as a quartet due to ortho and meta coupling, thereby establishing that one of the naphthalene rings contained three hydrogen atoms. The doublet at τ2.96 (1H) was attributed to one of the two protons in a meta relationship in the other ring. Clearly, only two situations are consistent with this evidence; the aromatic hydroxyl group must be placed at either C-5 or C-8. If, however, the hydroxyl was placed at C-8 the proton H-9 would be expected to appear as a doublet at high field. This was not the case and the aromatic hydroxyl group was therefore assigned to C-5, an assignment consistent with other members of the series.

The mass spectrum supported the proposed structure (XXX). The fragmentation pattern was basically the same as for other members of this series with the exception of


m/e
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
Relative Abundance
0
50
100
OH
OH
O


m/e 184 [3]
m*
m/e 172 [32]
m/e 201 [4]
m*
m/e 216 [100]
m/e 198 [15]
m/e 173 [7]
m/e 188 [8]
m/e 131 [14]
m*
m/e 115 [24]
m/e 187 [56]
m/e 171 [44]

SCHEME 13

a peak at $(P-18)^+$ due to loss of H_2O. The structural assignments in Scheme 13 are an attempt to rationalise all the observed data. Metastables at 187, 162 and 137 mass units correspond to the fragmentations P-15, P-29 and P-44 respectively but a concerted mechanism could only be postulated for the loss of a methyl group. However, observations in the mass spectra of several organic compounds indicate that metastable ions do not constitute conclusive evidence for one-step decomposition processes or elimination of structural entities. It appears that metastable ions may arise from two-step or possibly even multi-step fragmentation reactions.[62,63]

A quantitative u.v. spectrum indicated that the extinction coefficients for $\lambda_{max.}$ (EtOH) of 3,5-dihydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran were comparable with those published for xanthorrhoein.[14]

Xanthorrhoein: 225, 244, 287, 298, 323, 338 mμ (4.70, 4.44, 3.67, 3.64, 3.40, 3.49)

D21 : 225.5, 245.5, 289, 301.5, 327, 339 mμ (4.72, 4.36, 3.73, 3.70, 3.47, 3.53).

The preceding evidence establishes with reasonable certainty the gross structure of the compound, nevertheless, two aspects of the work require further

attention. In the first instance, the positioning of the aromatic hydroxyl group at C-5 needs to be fully substantiated by appropriate synthetic work. Here, the previous work on the synthesis of 1-oxaphenalene systems by Salahud-Din[14] and O'Brien and Smith[64] will no doubt prove useful. Secondly, the stereochemistry of the compound is of particular interest as it contains two adjacent asymmetric centres. The coupling constant $J_{2,3}$ = 8.5 c/s indicates that the protons on C-2 and C-3 have a trans-configuration i.e. the stereochemistry of the molecule is either 2S,3R or 2R,3S. If we assume that the configuration at C-2 is the same as in xanthorrhoeol the absolute configuration is 2S,3R (XXXI). In the event of more material becoming available this could be confirmed by exhaustive ozonolysis of the naphthopyran and subsequent isolation of 2,3-dihydroxybutyric acid, or a suitable derivative, for comparison with authentic samples.

3,5-dihydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran is unstable to light and air and discolours markedly in solution with acetone. It is the fifth naphthopyran to be isolated from a natural source and the fourth from a Xanthorrhoea resin.

An examination of the u.v., i.r. and p.m.r. absorption spectra of the neutral fraction (D3) indicated that it consisted of a complex mixture of aromatic esters. One component was identified, by qualitative gas chromatography, as methyl cinnamate.

(b) Sticky Resin

A small amount of the fresh yellow resin from the leaf bases was fractionated on the basis of acidity and the fractions thereby obtained were compared by t.l.c. with those from the brittle resin. No significant difference in the distribution of components was observed. The carbonate-insoluble fraction (the major fraction) was extracted with cold dilute sodium hydroxide and the alkali-soluble portion subjected to column chromatography on silicic acid. This resulted in the isolation and characterisation of small quantities of 5-hydroxy-2-methyl-2H,3H-naphtho [1,8-b,c] pyran and xanthorrhoeol. Several chalcones were also present in trace amounts and one of these, m.p. 128-130°, was tentatively identified by mass spectrometry as 2', 4'-dihydroxy-3,4-dimethoxychalcone, i.e. a dimethyl ether of butein (lit.[78] 127-128°).

(c) Inflorescence Material

Inflorescence material (scape and spike) was collected in the flowering season (mid-November) when the spike consisted of hundreds of small, cream coloured florets on a fleshy stem. Many of the spikes, particularly those that were more developed, showed signs of insect infestation. The insect primarily responsible for this appeared to be Hylaletis latro (Zell.).[65]

The plant material damaged by this insect was not examined in detail, however, it is worthy of note that the gelatinous material which lined the holes bored by the larvae contained a substance exhibiting a u.v. spectrum very similar to that of 5-hydroxy-2-methyl-2H, 3H-naphtho [1,8-b,c] pyran. Subsequent work suggested that naphthopyrans were absent from material free of infestation, so whether these compounds are produced by the plant or the insect is open to conjecture. This will be discussed in a later section but at this point it should be noted that both the insect species H.latro and the methyldihydronaphthopyran class of compound appear to be found only in association with Xanthorrhoea species.

Undamaged inflorescences were macerated and extracted with ethanol in the normal way and the ether-soluble portion of the extract, less than 0.5 per cent of the total undried weight of plant material,* was divided into bicarbonate-soluble, carbonate-soluble and carbonate-insoluble fractions.

The bicarbonate-soluble fraction was screened for the presence of aromatic carboxylic acids by t.l.c. Caffeic and p-coumaric acids were detected, while cinnamic acid, a common constituent of Xanthorrhoea resin extract, appeared to be absent.

Carboxylic Acids in the Inflorescence of X.australis

Rf values (Silica gel G : 0.2 mm)

Solvent	p-coumaric acid	caffeic acid
Benzene-dioxan-acetic acid (90:25:4)	0.50	0.30
Benzene-methanol-acetic acid (90:16:8)	0.42	0.32
Toluene-methylformate-formic acid (50:40:10)	0.48	0.50

* The major portion of the extract consists of carbohydrates. (ca. 60% of dry weight)[66]

Examination of carbonate-soluble and carbonate-insoluble fractions revealed that each contained one major component. Though obviously different (by t.l.c.), these compounds were similar in that they were both orange coloured in visible and u.v. light and developed a violet-red colouration on being sprayed with dilute ethanolic potassium hydroxide. The carbonate-insoluble compound, Rf = 0.69 (Silica gel G; $CHCl_3$), was identified as chrysophanic acid by comparison with an authentic sample. The carbonate-soluble compound, Rf = 0.10 (Silica gel G; $CHCl_3$), was designated "AQ" and subjected to further examination.

AQ: 1,8-dihydroxy-3-hydroxymethylanthraquinone. [aloe-emodin]

Purified by chromatography and recrystallised from ethanol, the compound AQ was obtained as orange-yellow plates m.p. 222-225°.

Analysis figures indicated a molecular formula of $C_{15}H_{10}O_5$ (Found: C, 66.53; H, 3.83; O, 30.6. Calc. for $C_{15}H_{10}O_5$: C, 66.7; H, 3.73; O, 30.6) and this was supported by a mass measurement of the molecular ion. (Found: 270.0527. Calc. for $C_{15}H_{10}O_5$: 270.0528).

The outstanding feature of the mass spectrum of the compound was the appearance of a major peak at m/e 241 (59 per cent) corresponding to the loss of 29 mass units from the molecular ion (and base peak). Mass measurements indicated that this was due to the loss of CHO.

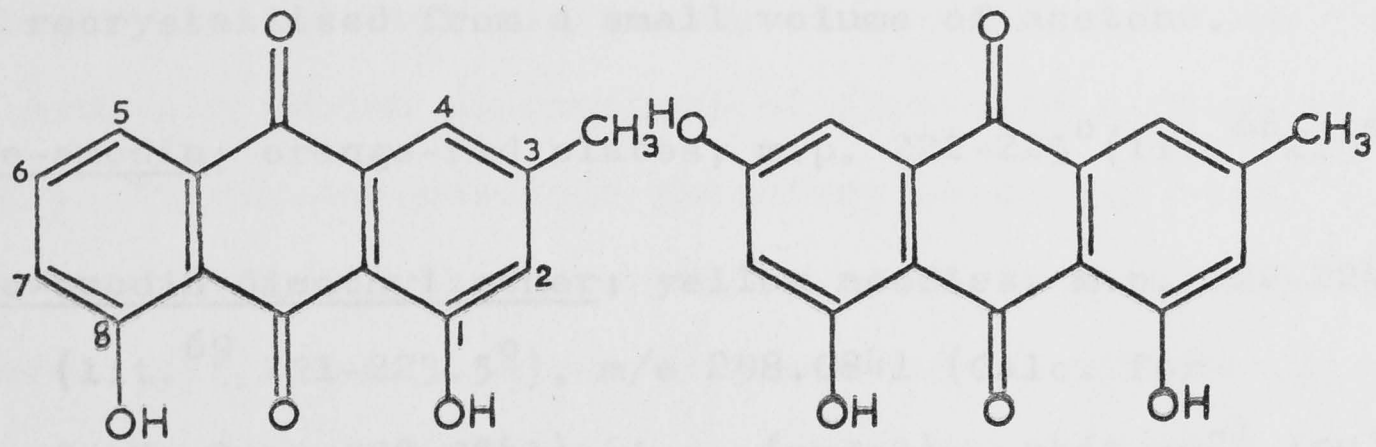
The u.v. spectra of AQ in neutral and basic solutions were virtually superimposable on those for chrysophanic acid under the same conditions. The compound was therefore clearly related to chrysophanic acid, but differing in that it contained an extra oxygen atom so placed as to not make a significant contribution to the absorbing chromophore. This evidence taken together with the fact that the compound readily lost 29 mass units, characteristic of benzyl alcohols,[67] strongly suggested that the compound was ω-hydroxy-chrysophanic acid (i.e. aloe-emodin). For comparison purposes the u.v. spectrum of the isomeric compound, emodin, was also considered.

U.v. Absorption Spectra of Some Anthraquinones

Compound	$\lambda_{max.}$ mμ(EtOH)(log ε)
Emodin[54]	222, 252, 265, 289, 437, 520-30
Chrysophanic Acid	225.5(4.58), 256(4.33), 277.5(4.05), 288(4.08), 430(4.02)
Aloe-emodin[69]	226(4.60), 256(4.33), , 288(4.05), 430(4.07)
AQ	226(4.56), 255.5(4.31), 277.5(3.95), 287.5(3.97), 430(3.98)

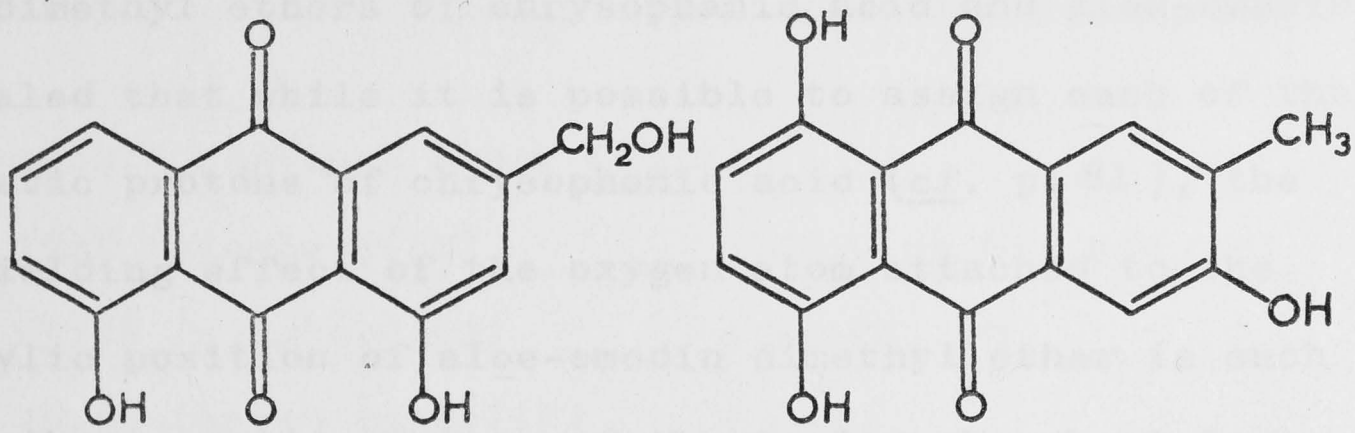
The i.r. absorption spectrum of AQ(nujol) was in agreement with that published for aloe-emodin.[69] In particular, the absorptions at 1675 cm^{-1} (medium) (non-conjugated CO) and 1630 cm^{-1} (strong)(conjugated CO) confirmed that the positions peri to one of the carbonyl groups were not hydroxylated.

Methylation of the anthraquinone with dimethylsulphate in acetone in the presence of potassium carbonate afforded a mixture of the di- and trimethyl



chrysophanic acid

emodin



aloe-emodin

isoemodin

ethers; the former being the major product. These were separated by column chromatography on magnesium oxide and recrystallised from a small volume of acetone.

aloe-emodin; orange-red plates, m.p. 222-225°(lit.[68] 223°).

aloe-emodin dimethyl ether; yellow needles, m.p. 222-224° (lit.[69] 221-223.5°), m/e 298.0841 (Calc. for $C_{17}H_{14}O_5$: 298.0841). i.r. (nujol): 3465 cm^{-1} (OH), 1665 cm^{-1} (non-conj. CO).

aloe-emodin trimethyl ether; red needles, m.p. 156-159° (lit.[69] 153-156°) m/e 312.0997 (Calc. for $C_{18}H_{16}O_5$: 312.0991). i.r. (nujol): 1670 cm^{-1} (non-conj. CO).

A comparison of the 100 Mc./sec. p.m.r. spectra of the dimethyl ethers of chrysophanic acid and aloe-emodin revealed that while it is possible to assign each of the aromatic protons of chrysophanic acid (cf. p. 81), the deshielding effect of the oxygen atom attached to the benzylic position of aloe-emodin dimethyl ether is such that the aromatic protons of the A-ring absorb at lower field making the overall range for the aromatic protons smaller. In addition, in the case of the aloe-emodin ether, solubility requirements necessitated the use of DMSO as solvent. This solvent was found to affect the

range of the aromatic protons in chrysophanic acid dimethyl ether (85 Hz in $CDCl_3$; 56 Hz in DMSO) and probably also contributed to the narrow range, 40 Hz, for the aromatic proton absorptions of aloe-emodin dimethyl ether. It was not possible therefore to assign each aromatic proton of this compound. Apart from this, however, all other spectral evidence obtained from the methylated anthraquinone AQ could be interpreted on the basis of the aloe-emodin structure.

Chrysophanic acid dimethyl ether (DMSO):

τ7.60 (singlet)(3H)	; CH_3 on C-3
τ6.12, 6.11 (doublet)(6H)	; CH_3O on C-1 and C-8
τ2.16 - τ2.72 (multiplet) (5H)	; aromatic protons

Aloe-emodin dimethyl ether (DMSO):

τ6.06 (singlet)(6H)	; CH_3O on C-1 and C-8
τ5.34 (singlet)(2H)	; Ar-CH_2-O
τ6.60 (broad band)(1H)	; O-H
τ2.18 - τ 2.58 (multiplet) (5H)	; aromatic protons

The above results showed conclusively that the anthraquinone "AQ" isolated from the inflorescence

extractives was aloe-emodin. The fact that this compound is completely extractable from solution in organic solvents with sodium carbonate solution is not consistent with the properties normally associated with anthraquinone derivatives. In addition, it should be noted that aloe-emodin forms an α-methoxy derivative on treatment with diazomethane.[69] Clearly, these properties should be borne in mind in the structural study of unknown anthraquinone compounds.

In 1899 Hesse[70] isolated from rhubarb a compound m.p. 212°, $C_{15}H_{10}O_5$, soluble in sodium carbonate solution and named it rhabarberone. A product of similar properties "isoemodin" was obtained by Eijken[71] in 1904 and he supposed it to be identical with rhabarberone.[72] Following a detailed examination of rhubarb, Tutin and Clewer[73] suggested in 1911 that rhabarberone and "isoemodin" were merely impure samples of aloe-emodin. Hesse,[74] however, regarded aloe-emodin and rhabarberone as distinctly different substances, the latter being soluble in sodium carbonate solution. Uchibayashi and Matsuoka[69] recently showed that aloe-emodin was definitely soluble in carbonate solution and they suggested that the identity of rhabarberone and "isoemodin" with aloe-emodin was all the more probable.

Re-investigation of the old preparations is impossible, but the fact that the occurrence of the compounds mentioned has never been reported since seems to support this suggestion.

Another aspect of this subject has given rise to a certain amount of confusion. In 1931 Keimatsu and Hirano[75] synthesised 2-methyl-3,5,8-trihydroxyanthraquinone and claimed it to be rhabarberone and "isoemodin" as it had the same melting point. It was not compared directly with natural materials, however, and this structure has now been discounted. It should be noted, however, that the names isoemodin and rhababerone (not rhabarberone) have been retained and currently refer to the compound 2-methyl-3,5,8-trihydroxyanthraquinone. They should not be confused with "isoemodin" (≡ aloe-emodin) mentioned above.

Aloe-emodin has not been previously isolated from a Xanthorrhoea species.

(d) Polymeric Material

The chemical nature of the polymeric material in Xanthorrhoea resin has long remained a mystery. It has been suggested[13] that as various cinnamyl and coumaryl esters (including cinnamyl-p-coumarate) have been isolated

the higher molecular weight material may simply represent the condensation of several C_6-C_3 units. On the other hand the isolation of the xanthorrhone dimers suggests that flavonoid C_6-C_3-C_6 units are most likely involved; either in association with one another or with C_6-C_3 units. A third possibility, that the material is a form of lignin, must also be considered.

In this investigation one somewhat unsuccessful attempt was made to separate the polymeric fraction. In 1950 Schuerch[76] developed a method of separating the components of hydrol lignin on the basis of differing molecular weights. His system of fractional precipitation, though fairly lengthy, was attractive from the point of view that there was little likelihood of altering the chemical composition of the bulk of the material. When, however, this system was applied to a sample of ether-insoluble resin obtained from X. australis results were not encouraging. All fractions were obtained as black viscous tars which on prolonged drying under vacuum formed brittle solids. One of these fractions was analysed by osmometric techniques and found to have an average molecular weight of 2595.

PART IV

AN EXAMINATION OF THE RESIN FROM XANTHORRHOEA HASTILE

A sample of yellow resin from X. hastile was obtained from the University of Sydney. The sample had been extracted with acetone from the original plant material by an earlier worker. Previous investigations of this resin had shown that while it contained relatively high proportions of cinnamic and coumaric acids, and various ether and ester derivatives of these, it was comparatively free of flavonoid material. The only compound of this class isolated was a chalcone, m.p. 154°, obtained from the methylated carbonate-soluble fraction.[18] It had not been fully characterised.

Since extractions of resin fractions with bicarbonate and carbonate solutions are never completely effective, and in this case we were dealing with reasonably high concentrations of cinnamic and coumaric acids (which are slow to go into bicarbonate solution) it was decided to modify the procedure in such a way that the carbonate-insoluble material was removed prior to extraction with bicarbonate. Apart from this, the method of initial fractionation on the basis of acidity followed that used

for X. preissii and X. australis. The fundamental difference in the composition of the red and yellow resins was reflected in the different solubility properties. The ether-soluble resin of X. hastile comprised -

Bicarbonate-soluble fraction	5 per cent
Carbonate-soluble fraction	12 per cent
Alkali-soluble fraction	79 per cent
Neutral fraction	4 per cent

The bicarbonate-soluble fractions was purified by column chromatography and shown to consist almost entirely of p-coumaric acid, m.p. 209-210°, and cinnamic acid, m.p. 132-133° (5:1). The physical and spectral properties of both samples were in complete agreement with those exhibited by authentic material. The coupling constants between the α- and β-protons (J = 16 c/s) showed that the compounds had a trans-configuration about the double bond.*

---

* McReavie[18] had previously isolated a compound, m.p. 88°, from X. hastile which she considered to be cinnamyl cinnamate. However, authentic trans-trans-cinnamyl cinnamate was found to have a m.p. 44° and it had then been considered that perhaps the material isolated had a cis double bond and that cis-cinnamic acid also occurred in the resin. There appeared to be no evidence to support this claim.

The carbonate-soluble fraction was chromatographed on silicic acid (20 per cent EtOAc-$CHCl_3$). Despite the earlier work on this resin, the material contained in several of the fractions was undoubtedly flavonoid. Recrystallisation of one of the fractions from aqueous alcohol resulted in the isolation of material, m.p. 192-193°, having physical can spectral properties identical to those of 5,7-dihydroxy-4'-methoxyflavanone previously isolated. Another fraction was found to contain a small quantity of a colourless crystalline compound m.p. 250° which appeared from u.v. and mass spectral measurements to be naringenin, i.e. 4',5,7-trihydroxyflavanone. This was confirmed by comparison (m.m.p. and t.l.c.) with a sample of this flavanone which had been isolated by Salahud-Din from X. preissii.[28]

A third and far more significant component of this portion of the resin was also identified as a flavanone. This substance, which represented 8.7 per cent of the carbonate-soluble fraction, appeared in several fractions and was designated as "H35".

H35: 4'-methoxy-5,7,3'-trihydroxyflavanone. [Hesperetin]

The pale yellow semi-crystalline material which comprised this fraction exhibited a u.v. absorption spectrum normally characteristic of flavanones. Though

easily crystallised as colourless needles from aqueous ethanol, five recrystallisations from this medium failed to improve the melting point of the substance beyond the range 197-199°.

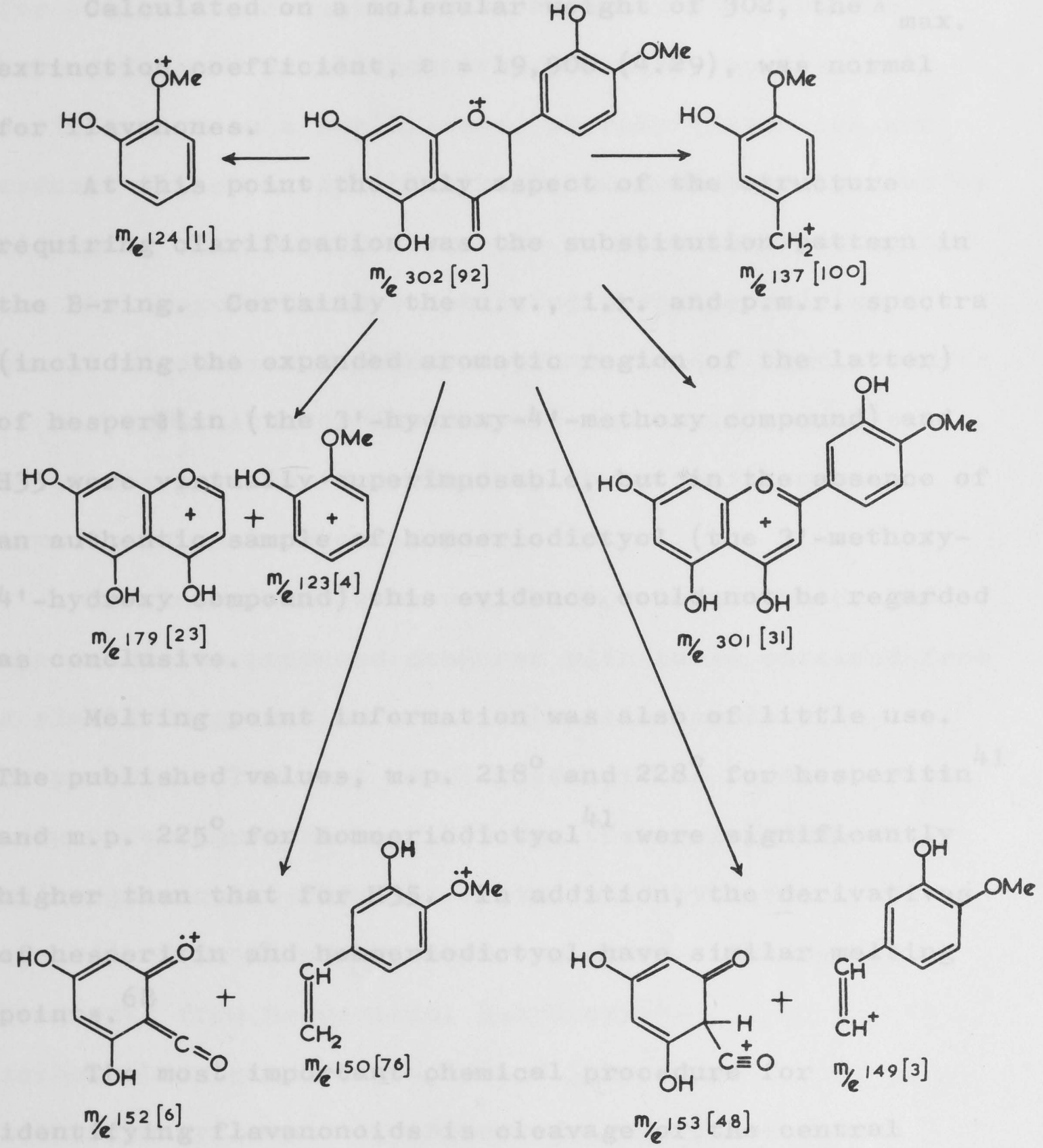
The purified material was examined by the usual spectroscopic methods. $\lambda_{max.}$ (EtOH) occurred at 288.5 mμ and 325 mμ (shoulder), bathochromic shifts of 40 and 20 mμ were observed on the addition of alkali[33] and aluminium chloride[34] respectively, and the addition of sodium acetate[35] caused the long wavelength shoulder to be resolved into a peak at 329 mμ. In the i.r. spectrum (nujol mull), the carbonyl absorption occurred at 1638 cm^{-1} and hydroxyl absorptions appeared as a sharp peak at 3500 cm^{-1} and a broad band at 3120 cm^{-1}. The compound gave a positive Shinoda test[36] and reduction with borohydride followed by acidification produced a magenta colour. There seemed little doubt on this basis that the compound was a derivative of 5,7-dihydroxyflavanone.

The p.m.r. spectrum of H35 was measured in deuteroacetone. The aliphatic protons on C-2 and C-3 produced the characteristic ABX pattern of a flavanone with a quartet at τ4.62 ($J_{2,3a}$ = 12.5 c/s; $J_{2,3e}$ = 3.6 c/s) due to the proton on C-2, and two one-proton

quartets at τ6.90 and τ7.29 ($J_{3e,3a}$ = 16.5 c/s) due to the C-3 protons. A sharp three-proton singlet at τ 6.18 came from methoxyl proton absorption and hydroxyl protons produced a sharp singlet at -τ2.12 (1H) and a broad band at τ6.86 which overlapped with the lower part of the multiplet. The aromatic protons on C-6 and C-8 appeared as a two-proton singlet at τ4.08.

The remaining part of the spectrum, that due to aromatic B-ring protons, proved notably significant. The proton absorptions appeared as two bands, at τ3.00 and τ3.08, they integrated for three protons thereby indicating a disubstitution pattern, and the general appearance of the peaks in an expanded spectrum suggested that they belonged to an AA'X system, i.e. the system found in a benzene ring substituted at C-1, C-3 and C-4.

The mass spectrum of the compound showed a molecular ion at m/e 302 (≡ $C_{16}H_{14}O_6$) and from this and the previous evidence it was concluded that the substance was a 5,7-dihydroxyflavanone substituted in the B-ring with hydroxyl and methoxyl groups; most likely at the C-3' and C-4' positions. The mass spectral fragmentation pattern (Scheme 14) supported this conclusion. [Note: Small peaks were observed at m/e 316(302 + 14) and m/e 167 (153 + 14) so there appeared to be a trace of the methyl ether of the compound also present].


HO
OMe
HO
O
OH
O
m/e 302 [92]
OMe
HO
m/e 124 [11]
OMe
HO
CH2+
m/e 137 [100]
HO
O
OH
OH
m/e 179 [23]
OMe
HO
m/e 123 [4]
OH
OMe
HO
O
OH
OH
m/e 301 [31]
HO
O
OH
m/e 152 [6]
OH
OMe
CH
CH2
m/e 150 [76]
HO
O
H
C≡O
OH
m/e 153 [48]
OH
OMe
CH
CH+
m/e 149 [3]

SCHEME 14

Calculated on a molecular weight of 302, the $\lambda_{max.}$ extinction coefficient, ε = 19,900 (4.29), was normal for flavanones.

At this point the only aspect of the structure requiring clarification was the substitution pattern in the B-ring. Certainly the u.v., i.r. and p.m.r. spectra (including the expanded aromatic region of the latter) of hesperetin (the 3'-hydroxy-4'-methoxy compound) and H35 were virtually superimposable, but in the absence of an authentic sample of homoeriodictyol (the 3'-methoxy-4'-hydroxy compound) this evidence could not be regarded as conclusive.

Melting point information was also of little use. The published values, m.p. 218° and 228° for hesperitin[41] and m.p. 225° for homoeriodictyol[41] were significantly higher than that for H35. In addition, the derivatives of hesperitin and homoeriodictyol have similar melting points.[68]

The most important chemical procedure for identifying flavanonoids is cleavage of the central pyran ring with hydroxyl ion and subsequent identification of the fragments produced. Classically, this is carried out either by fusion of solid flavonoid with potassium hydroxide or by heating in dilute alkali

for 2-6 hr.[56] Recently, however,Hurst and Harborne[77] have developed an alternative procedure, particularly satisfactory on a small scale, whereby flavonoids are reductively cleaved with alkali and sodium amalgam under nitrogen. The B-ring fragments produced by this method are the appropriately substituted phenylpropanols, benzyl alcohols and phenylpropionic acids. The products of the reductive degradation are then examined by t.l.c. on silica gel and identified by their position on a two-dimensional chromatoplate.

Samples of H35 were degraded by both methods and the fragments produced compared with those obtained from a similar degradation of an authentic sample of hesperetin. The most readily identified product of the reductive degradation of H35 was the A-ring fragment, phloroglucinol. This was accompanied by a trace of resorcinol produced by dehydroxylation. The B-ring fragments from hesperitin, 3-hydroxy-4-methoxyphenylpropionic acid and 3-hydroxy-4-methoxyphenylpropanol, appeared to correspond with those from H35.

Gentle potassium hydroxide fusion of both hesperetin and H35 produced colourless crystalline material, having m.p. 255° (purified by sublimation),

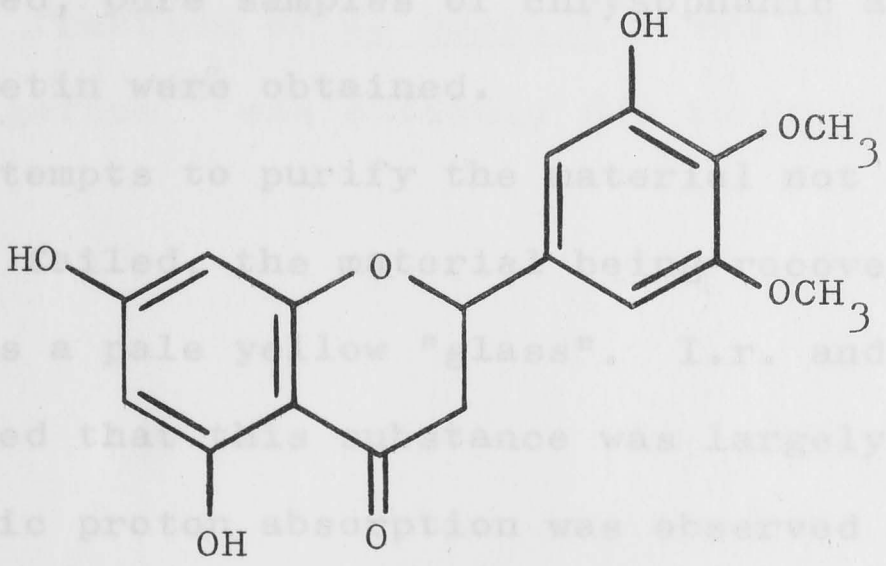
which was indistinguishable by the usual physical and spectral methods from authentic isovanillic acid.

It was concluded on the basis of the foregoing evidence that the compound under examination was hesperitin and that the slightly depressed melting point was due to contamination by its methyl ether. (It was noted, however, that the p.m.r. spectrum showed the methoxyl proton absorptions as a clean sharp singlet with a correct integration, so only a small amount of such an impurity could be involved.)

The isolation of hesperitin from this fraction of the resin probably provides the key to the structure of the "yellow compound, m.p. 154°" isolated by McReavie[18] following methylation of the carbonate-soluble fraction from a sample of X. hastile resin. The chalcone formed from the trimethylether of hesperetin, i.e. 2'-hydroxy-3,4,4',6'-tetramethoxychalcone, has a melting-point of 148-150°.[79] McReavie had, in fact, considered such a structure, but had dismissed it on the grounds of m.m.p. depression with an "authentic" sample.

Hesperetin was the first flavanone disubstituted in the B-ring to be isolated from a Xanthorrhoea resin.

An examination of the fractions which were eluted immediately after hesperetin showed that they contained mixtures of flavanones and chalcones. A 15eV mass spectrum of a yellow crystalline fraction, H48 (flavanone : chalcone = 1:1 by u.v.), showed molecular ions at m/e 256,272,286, 302 and 332 with relative abundances in the ratio 4:5:2:7:1 respectively. Since compounds of this class do not readily lose 16 or 14 mass units by normal fragmentation it was reasonable to postulate that these compounds represented simple hydroxy and methoxy derivatives of flavanone and chalcone. The compound of highest molecular weight (m/e 332) would appear to have a structure such as (XXXII) so flavanones trisubstituted in the B-ring are probably present in the resin also.



XXXII

The carbonate-insoluble fraction of the resin was next examined. Qualitative t.l.c. with a variety of solvent systems quickly established that this resin fraction was significantly different from the corresponding fractions from red resins. It contained none of the naphthopyran compounds and only small traces of flavonoids. In contrast to the multitude of reasonably discrete bands produced on t.l.c. plates by red resin extracts, this fraction "streaked" badly under all systems used. Chromatography was therefore considered impractical at this stage and the fraction was extracted with ice-cold sodium hydroxide solution.

The alkali-soluble fraction was extracted with several portions of warm benzene and the extract chromatographed on silicic acid. From the fractions collected, pure samples of chrysophanic acid and sakuranetin were obtained.

Attempts to purify the material not extracted with benzene failed, the material being recovered in most cases as a pale yellow "glass". I.r. and p.m.r. spectra indicated that this substance was largely aromatic. No aliphatic proton absorption was observed although a small p.m.r. band about τ6.1 probably corresponded to methoxyl absorption. Mass spectral measurements indicated

that the material was a complex mixture of high molecular weight substances; the molecular ions occurring at greater than 600 mass units. Attempts at degrading the material, e.g. by permanganate oxidation, have met with little success, and to date only small fragments such as benzoic acid have been identified.

The neutral fraction of the resin was purified by column chromatography on low activity neutral alumina and silicic acid. The resulting fractions were examined by u.v. and i.r. spectroscopy and by gas chromatography on Carbowax and XE60 columns and found to consist almost entirely of cinnamyl alcohol and methyl cinnamate in the ratio 1 : 5. There was no evidence to suggest that methyl-p-methoxy-cinnamate was present, and it now seems likely that the appearance of this compound in the neutral fraction of X. hastile resin in a previous investigation[18] was entirely due to in vitro methylation.

PART V

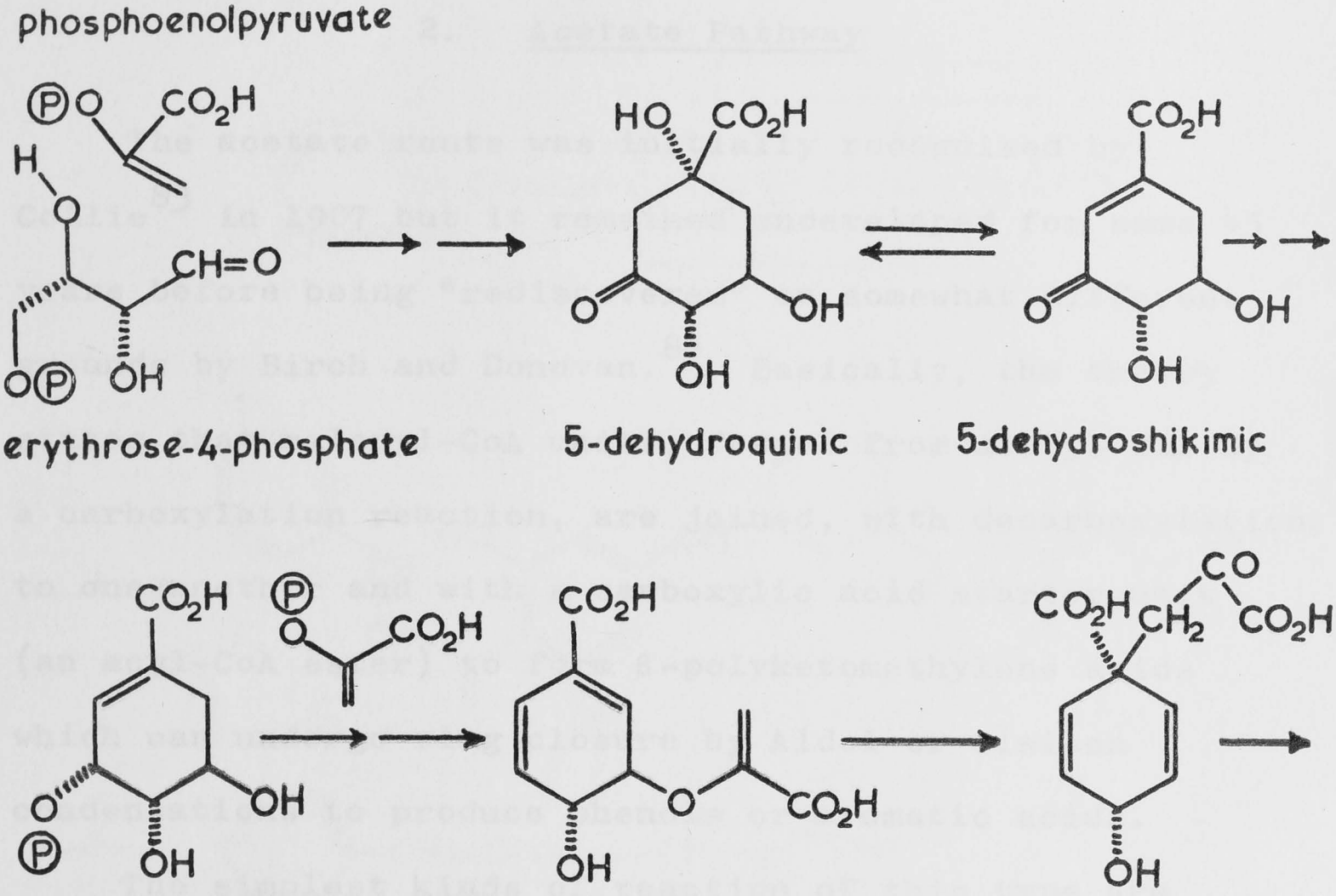
BIOSYNTHETIC CONSIDERATIONS

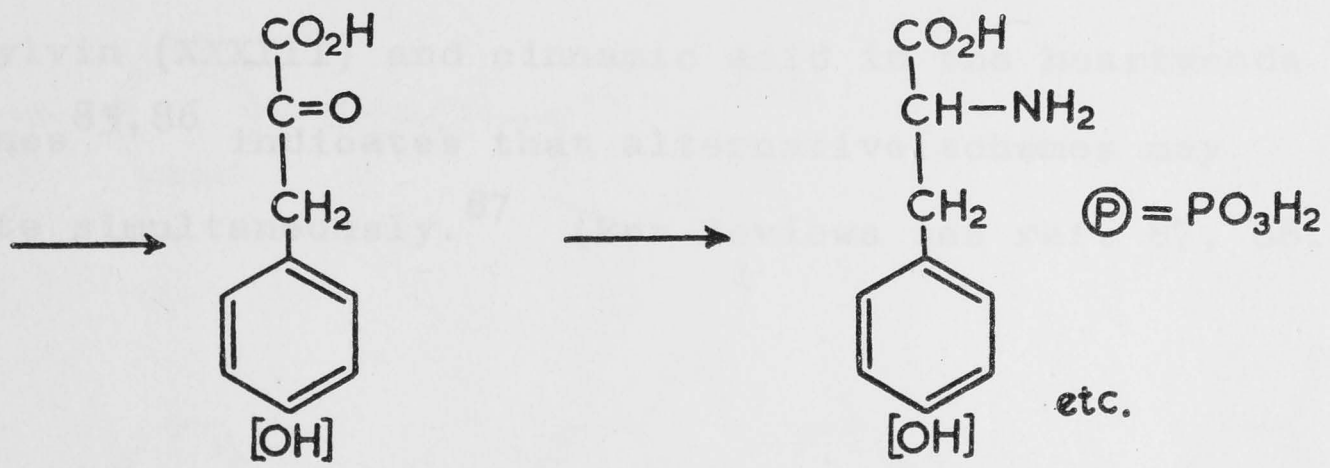
The major biosynthetic routes are now firmly established and present-day excursions into the field of natural products are usually accompanied by a survey of the relevant biogenetic propositions. Postulates are then made regarding the probable biogenetic origin of the compounds being considered and the relationships between them defined in terms of their common precursors.

In the current investigation we are concerned largely with aromatic compounds, and the pathways to be considered here are based on shikimic acid or on acetyl or malonyl coenzyme units.

1. Shikimic acid Pathway

Shikimic acid is the key compound in the biogenetic route which has taken shape as a result of the work of Davis and collaborators on the mutants of micro-organisms.[80] It is seen to arise through the condensation and subsequent cyclisation of D-erythrose-4-phosphate and phosphoenolpyruvate and is therefore directly related to sugar metabolism. Shikimic acid can condense with a further molecule of phosphoenolpyruvate to produce chorismic acid which can proceed via prephenic acid[81] to give various C_6C_3 compounds. (cf. Scheme 15) (For Reviews see ref. 82).


phosphoenolpyruvate
erythrose-4-phosphate
5-dehydroquinic
5-dehydroshikimic
shikimic acid phosphate
chorismic acid
prephenic acid

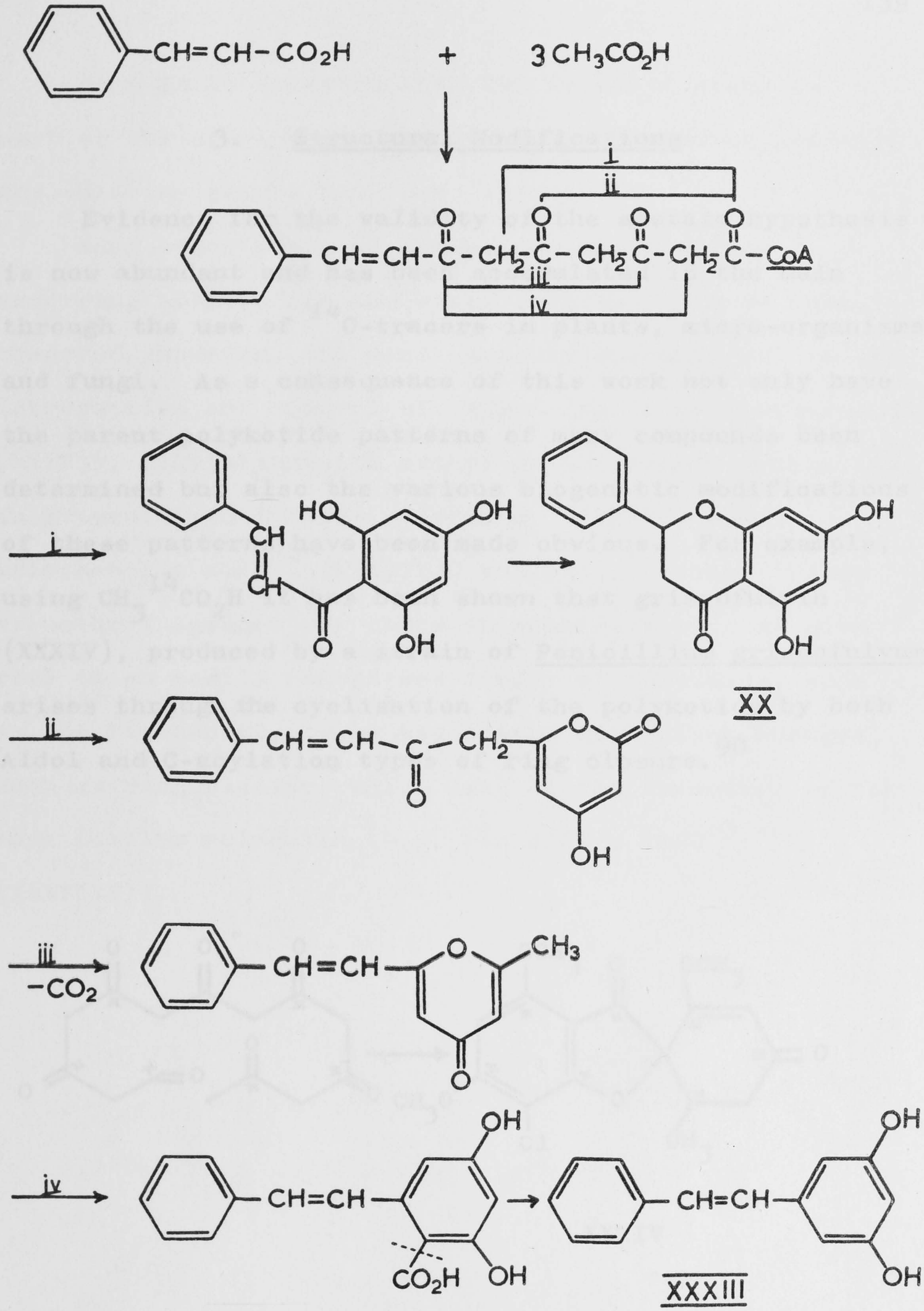

[OH]
[OH]
etc.
Ⓟ = PO3H2

SCHEME 15

2. Acetate Pathway

The acetate route was initially recognised by Collie[83] in 1907 but it remained undeveloped for some 45 years before being "rediscovered" on somewhat different grounds by Birch and Donovan.[84] Basically, the theory states that malonyl-CoA units, formed from acetyl-CoA by a carboxylation reaction, are joined, with decarboxylation, to one another and with a carboxylic acid starter unit (an acyl-CoA ester) to form β-polyketomethylene acids which can undergo ring closure by Aldol or Claisen condensations to produce phenols or aromatic acids.

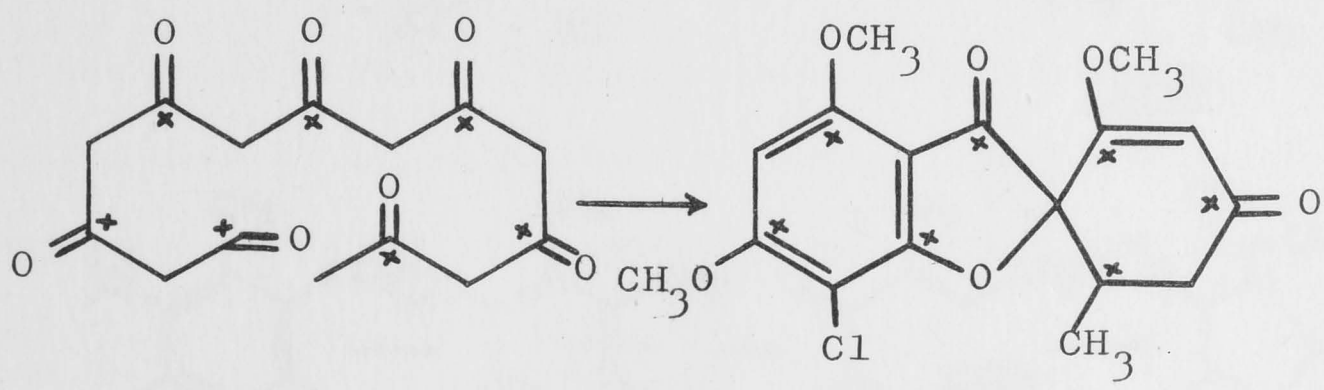
The simplest kinds of reaction of this type are illustrated in Scheme 16. The co-occurrence of flavonoids, e.g. pinocembrin (XX), derivatives of pinosylvin (XXXIII) and cinnamic acid in the heartwoods of pines[85,86] indicates that alternative schemes may operate simultaneously.[87] (For Reviews see ref. 87, 88, 89).


CH=CH-CO2H
+
3 CH3CO2H
i
ii
iii
iv
CH=CH-C-CH2-C-CH2-C-CH2-C-CoA
HO
OH
CH
CH
O
OH
O
OH
O
OH
XX
CH=CH-C-CH2
CH3
-CO2
CO2H
OH
CH=CH
OH
XXXIII

SCHEME 16

3. Structural Modifications

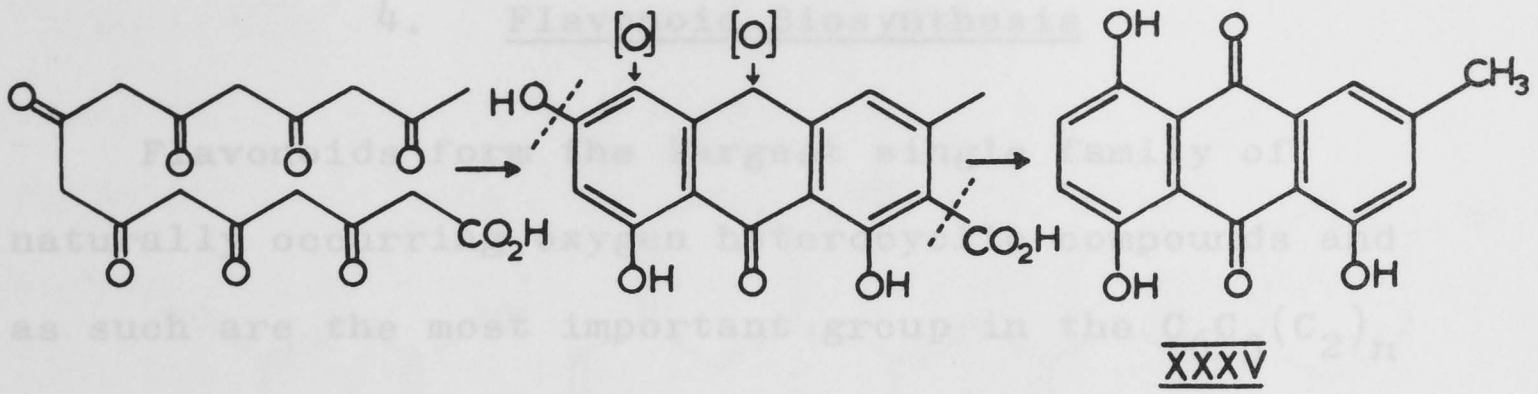
Evidence for the validity of the acetate hypothesis is now abundant and has been accumulated in the main through the use of ^{14}C-tracers in plants, micro-organisms and fungi. As a consequence of this work not only have the parent polyketide patterns of many compounds been determined but also the various biogenetic modifications of these patterns have been made obvious. For example, using $CH_3{}^{14}CO_2H$ it has been shown that griseofulvin (XXXIV), produced by a strain of Penicillium griseofulvum, arises through the cyclisation of the polyketide by both Aldol and C-acylation types of ring closure.[90]



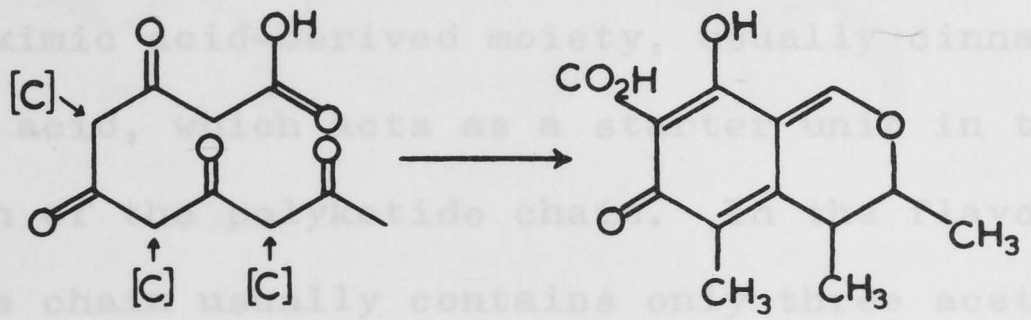
XXXIV

Here it is apparent that the C-methyl group is part of the original polyketide chain while the O-methyl and chlorine groups have been introduced.

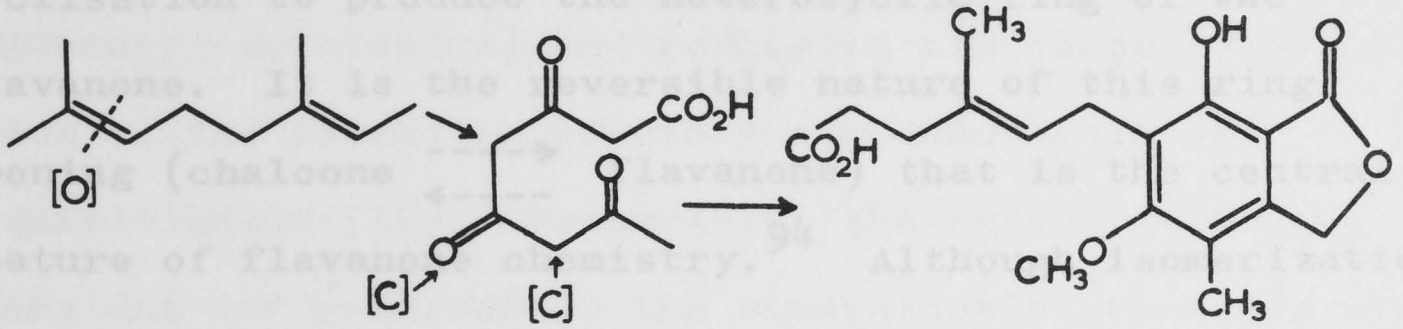
Many other kinds of structural modification occurring both before and after cyclisation have been observed, however, the most commonly encountered are the introduction and removal of oxygen (as in helminthosporin[91] (XXXV)), alkylation with methyl or isoprenoid groups (as in griseofulvin (XXXIV), citrinin[92] (XXXVI) and mycophenolic acid[89] (XXXVII)) and halogenation. These structural changes may occur in combinations that give rise to an easily recognised family of compounds, such as the flavonoids, or be associated with other changes, such as ring fission, which lead to the formation of far more complex molecules (e.g. penicillic acid[93] (XXXVIII)).



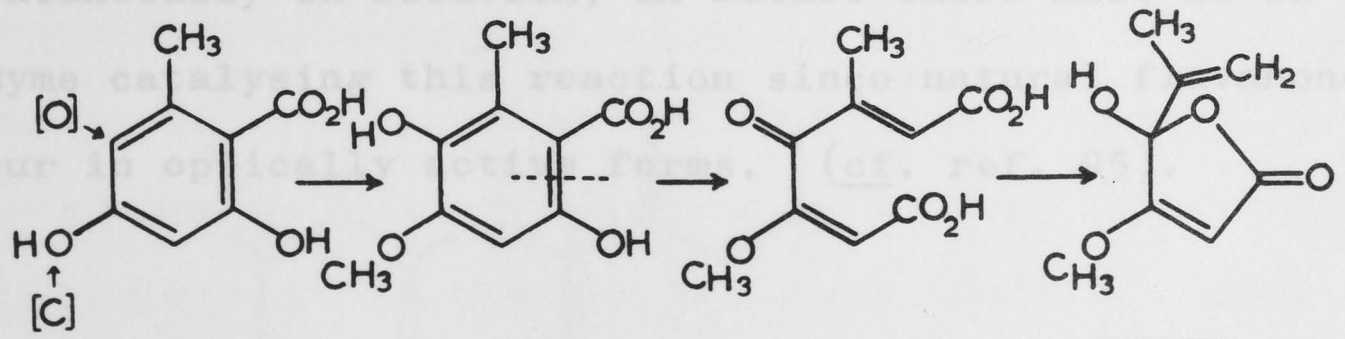
XXXV



XXXVI



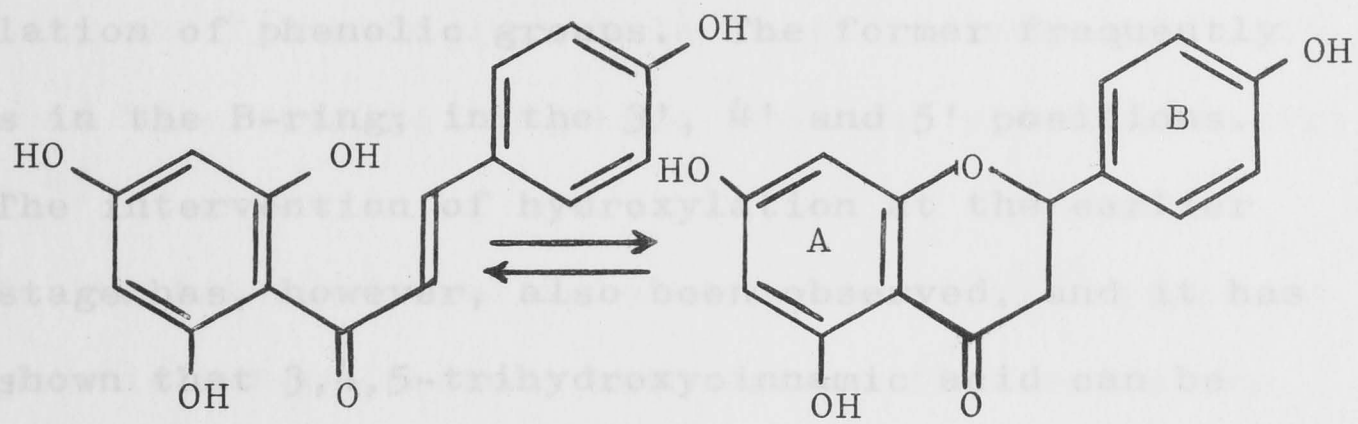
XXXVII



XXXVIII

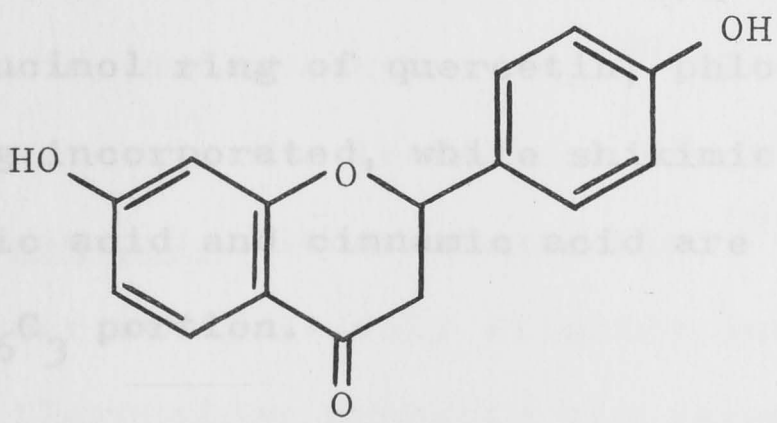
4. Flavonoid Biosynthesis

Flavonoids form the largest single family of naturally occurring oxygen heterocyclic compounds and as such are the most important group in the $C_6C_3(C_2)_n$ class of compounds. This class is characterised by its C_6C_3 shikimic acid-derived moiety, usually cinnamic or coumaric acid, which acts as a starter unit in the formation of the polyketide chain. In the flavonoid group the chain usually contains only three acetate units and these cyclise to produce a phloroglucinol ring as in the chalcone (XXXIX). This chalcone can undergo further cyclisation to produce the heterocyclic ring of the flavanone. It is the reversible nature of this ring opening (chalcone $\overset{\longrightarrow}{\underset{\longleftarrow}{\text{----}}}$ flavanone) that is the central feature of flavanone chemistry.[94] Although isomerization of most chalcones to the corresponding flavanones occurs spontaneously in solution, in nature there must be an enzyme catalysing this reaction since natural flavanones occur in optically active forms. (cf. ref. 95).



XXXIX

Modifications to the structure, of the kinds previously outlined, are frequently observed. In the open polyketide chain form, C-methylation (probably due to S-adenosyl-methionine) and reduction can occur. The latter leads to the formation of deoxy compounds such as liquiritigenin (XL). Presumably, the removable oxygen atoms are not required in the biosynthetic processes of stabilising or cyclising the chain.



XL

The most common structural changes occur after the formation of the A-ring and usually involve the introduction of additional hydroxyl groups and methylation of phenolic groups. The former frequently occurs in the B-ring; in the 3', 4' and 5' positions.

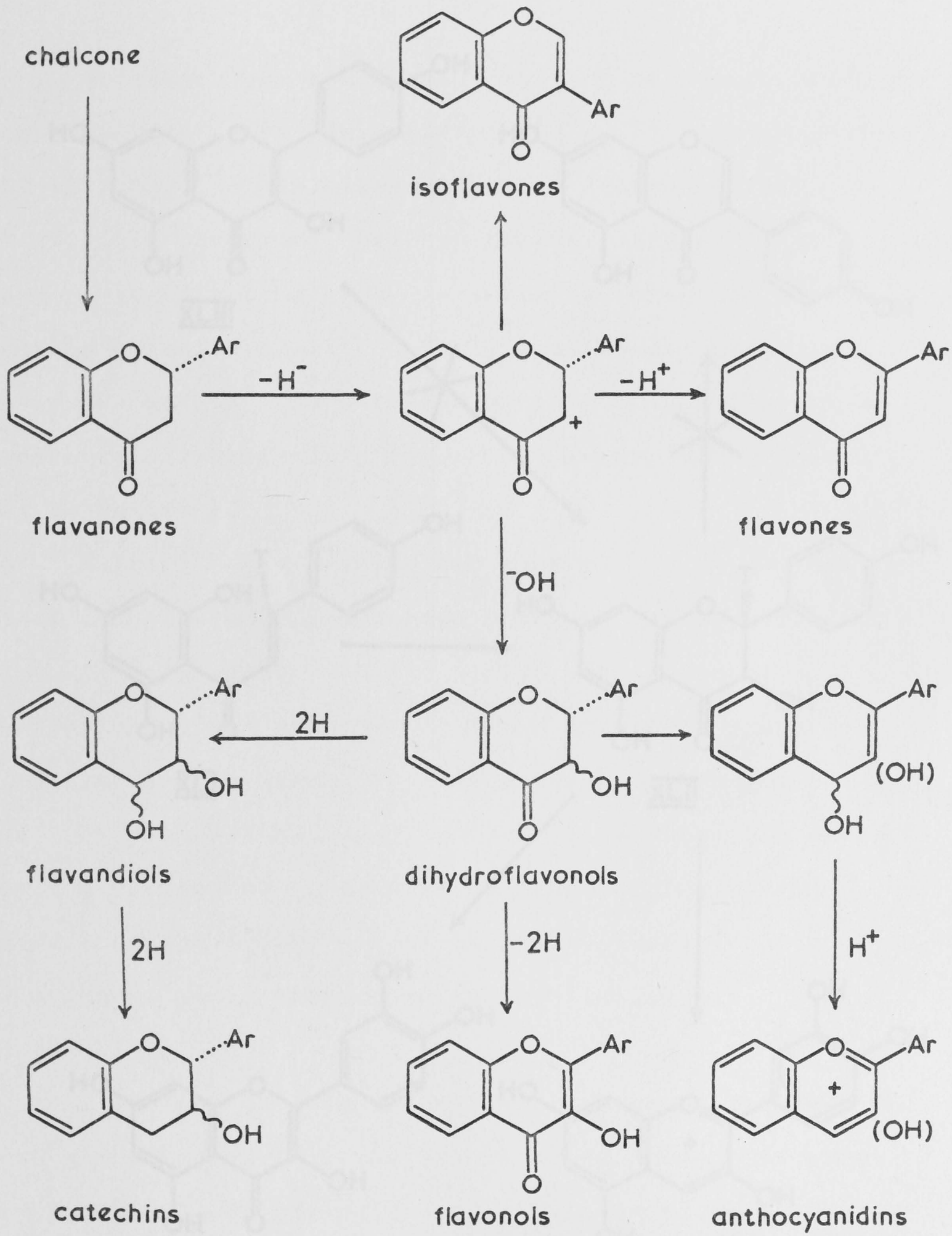
The intervention of hydroxylation at the earlier C_6C_3 stage has, however, also been observed, and it has been shown that 3,4,5-trihydroxycinnamic acid can be directly converted into delphinidin.[96] It appears then that B-ring hydroxylation can operate by two routes, either in different plants or even in the same plant if there is a sufficient lack of enzyme specificity.

Up to the point of chalcone formation the biogenetic pathway is well established, evidence being drawn from structure comparisons and precursor incorporation studies. Perhaps the most notable work in this regard was that done by Watkin, Underhill and Neish[97] on buckwheat (Fagopyrum tartaricum). They showed that labelled acetate is incorporated selectively into the phloroglucinol ring of quercetin, phloroglucinol itself not being incorporated, while shikimic acid, phenylalanine, p-coumaric acid and cinnamic acid are specific precursors of the C_6C_3 portion.

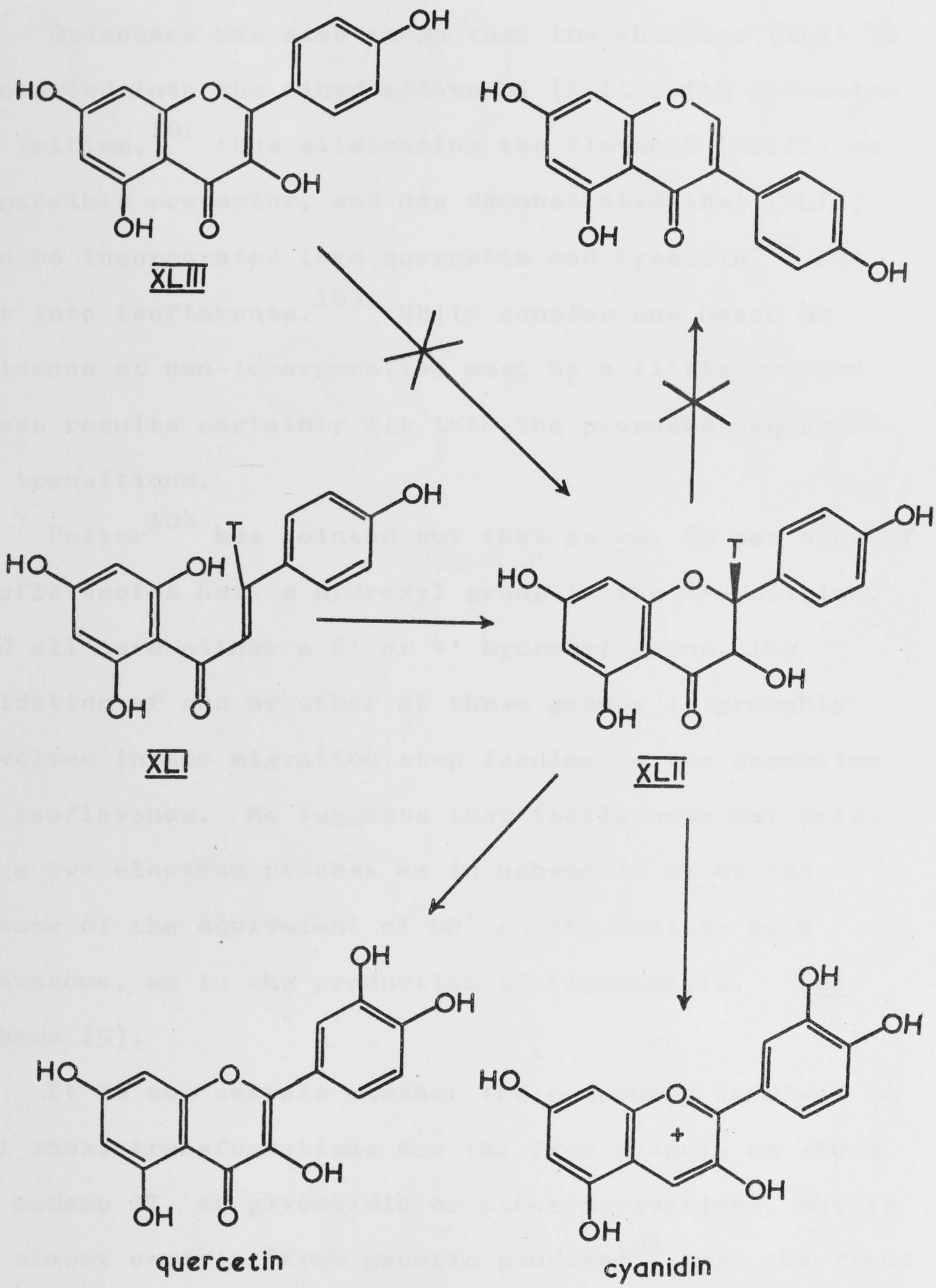
Evidence for the relationship between the flavonoids is generally far less direct. Chemotaxonomic evidence suggests a generic relationship between dihydroflavonols, flavonols and flavandiols, while most flavonoids with a reduced heterocyclic ring have the same stereochemistry at C-2.[45]

On the basis of available evidence Birch[98] proposed a biogenetic scheme whereby the various flavonoids are differentiated. (cf. Scheme 17). The key reaction in the scheme is the oxidation of flavanones at the enolic position. This is followed by reaction of the cation with a hydroxyl ion to give dihydroflavonols etc. or by proton elimination to give flavones. Isoflavone formation appears as an alternative to flavone formation and involves the migration of the B-ring aryl group.

Recent experimental work by Grisebach et al. indicates that this scheme is largely correct. (-) Naringenin (a flavanone) is specifically incorporated, with hydroxylation of ring B, into quercetin (a flavonol) and cyanidin (an anthocyanidin).[99] It is also incorporated into the isoflavone biochanin-A[99] and, in Cammelia sinensis, into epicatechin.[100] (+) Naringenin, on the other hand, is only slightly incorporated into any of these flavonoids; probably via racemization.


chalcone
isoflavones
Ar
flavanones
-H⁻
-H⁺
flavones
⁻OH
2H
flavandiols
dihydroflavonols
(OH)
OH
2H
-2H
H⁺
catechins
flavonols
anthocyanidins

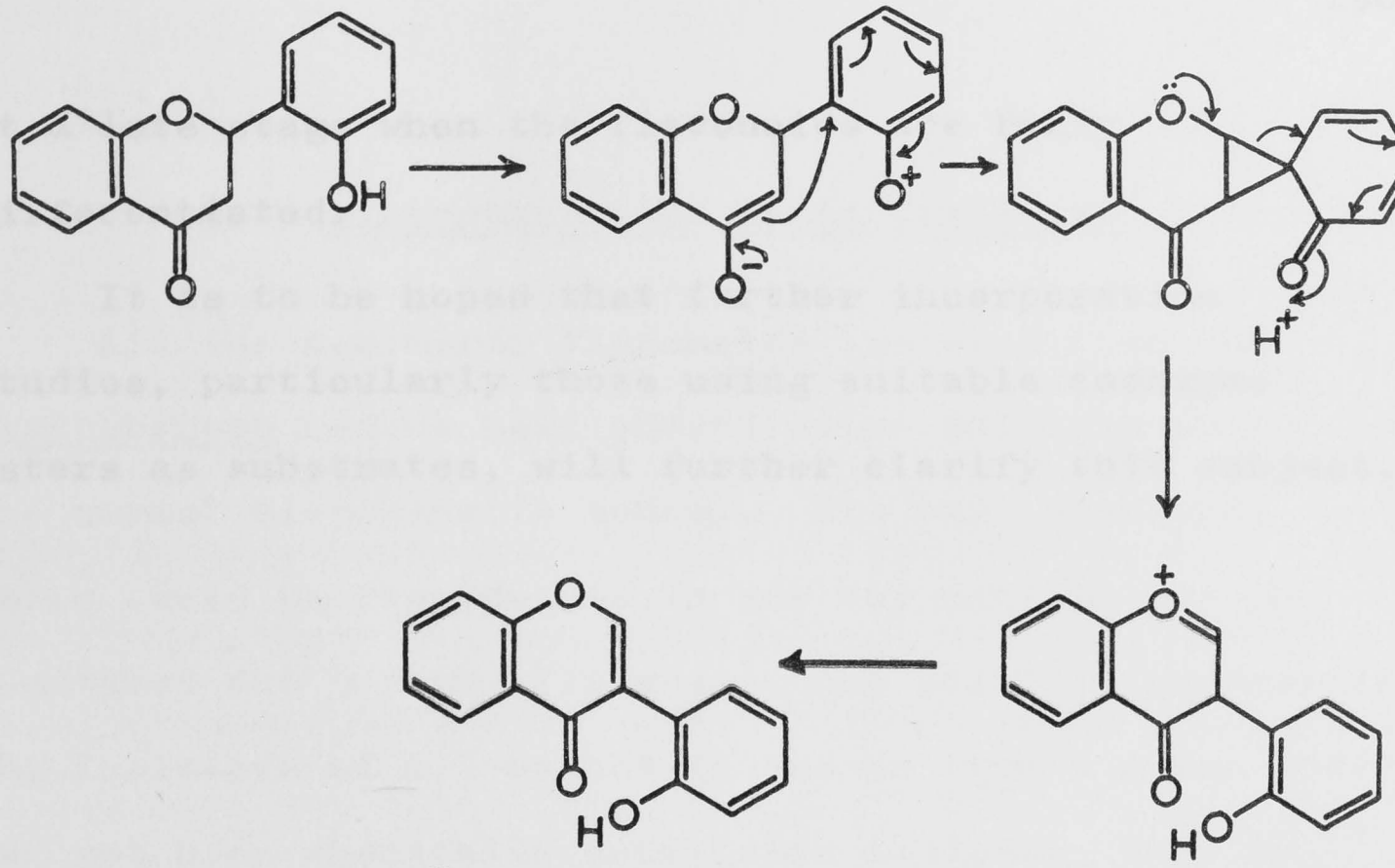
SCHEME 17


XLIII
XLI
XLII
quercetin
cyanidin

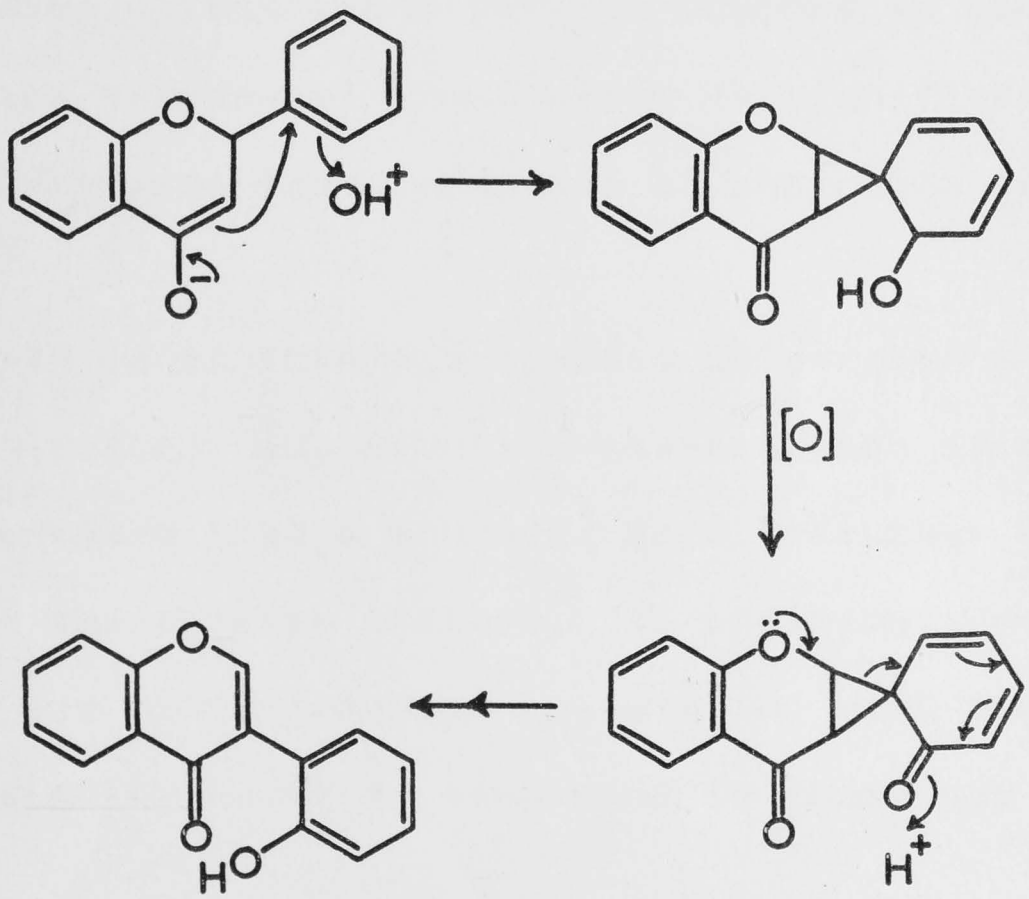
Grisebach has also shown that the chalcone (XLI) is converted into the dihydroflavonol (XLII) with retention of tritium,[101] thus eliminating the flavonol (XLIII) as a possible precursor, and has demonstrated that (XLII) can be incorporated into quercetin and cyanidin[102] but not into isoflavones.[103] While conclusions based on evidence of non-incorporation must be a little guarded these results certainly fit into the proposed sequence of transitions.

Pelter[104] has pointed out that as ca. 60 per cent of isoflavonoids have a hydroxyl group in the 2'-position, and all have either a 2' or 4' hydroxyl group, the oxidation of one or other of these groups is probably involved in the migration step leading to the formation of isoflavones. He suggests that isoflavones may arise by a two electron process as in Scheme 18 or by the attack of the equivalent of OH^+ on the enolate of a flavanone, as in the production of flavanonols. (cf. Scheme 19).

It is not certain whether the compounds involved in all these transformations are the free phenols as shown in Scheme 17, or glycosidic or other derivatives, but it is almost certain (from genetic studies)[98] that the final pattern of glycosylation, methylation etc. is established



SCHEME 18



SCHEME 19

at a late stage when the flavonoids are fully differentiated.

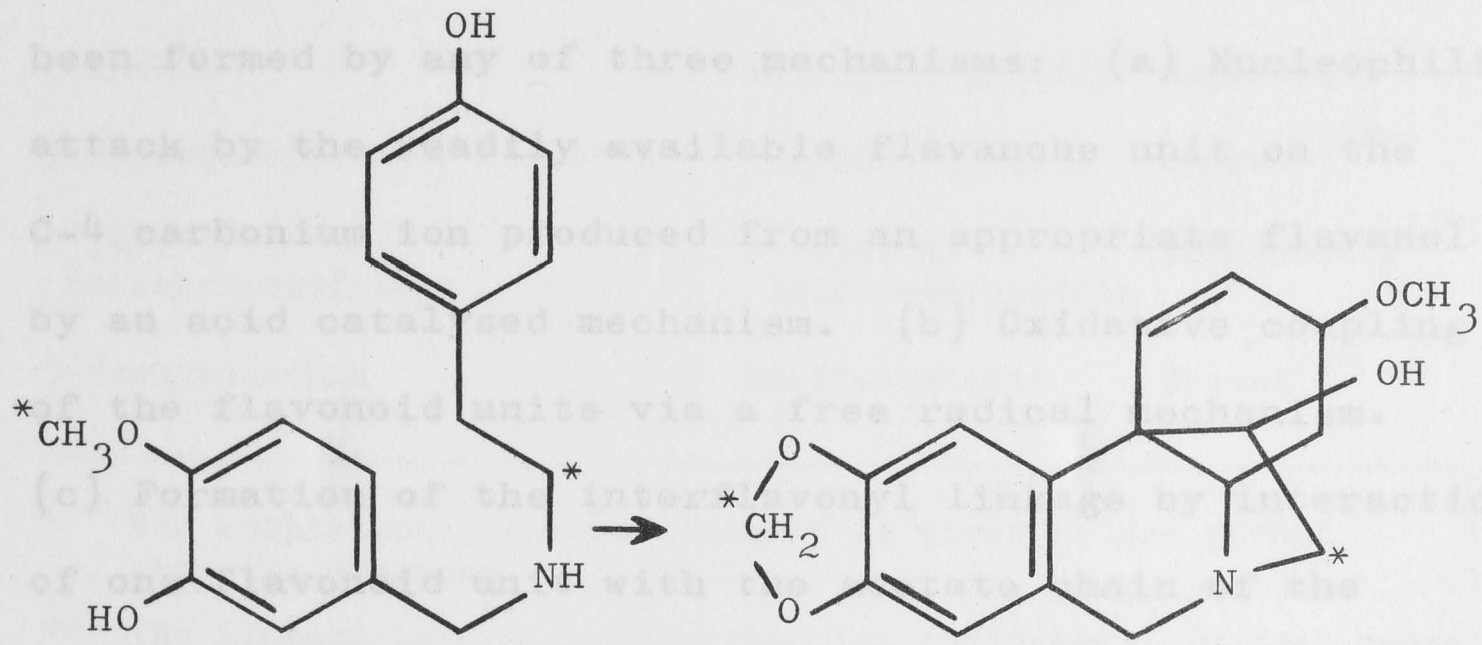
It is to be hoped that further incorporation studies, particularly those using suitable coenzyme esters as substrates, will further clarify this subject.

5. Biosynthesis of Resin Components

All the monomeric flavonoids isolated from the Xanthorrhoea resins have substitution patterns which fit the normal biosynthetic scheme. The only compounds which could be regarded as in any way unusual are the 5-methoxy and 5-deoxy flavanones and possibly hesperetin. The isolation of a 5-methoxyflavanone from a resin which had not been chemically methylated indicates that in this case methylation, like deoxygenation, has occurred at an early stage in the biosynthetic pathway; certainly before the formation of the flavanone in which the hydroxyl group would be protected to a large extent by hydrogen bonding. This lends further support to the belief that the 5-hydroxyl group plays no significant part in the enzymatic transformation of chalcones to flavanones.[59]

Hesperetin is of interest because it carries a 3'-hydroxy-4'-methoxy substitution pattern when it might have been predicted that a coumaric acid precursor would have produced the reverse pattern. In addition, compounds of this type are often found in association with the corresponding methylenedioxy compounds so these may also

be present in the resin. The hypothesis that methylenedioxy groups could be derived biogenetically from ortho-methoxyphenols was first mentioned by Sribney and Kirkwood,[105] and has also been discussed by Birch.[88a] More recently, during a study of the biosynthesis of Amaryllidaceae alkaloids, Barton, Kirby and Taylor[106] have shown that the phenol (XLIV) can be efficiently incorporated into haemanthamine (XLV). Their experiments showed that for this case, at least, the methylenedioxy group was formed by oxidative cyclisation of methoxyl to methylenedioxy rather than by demethylation followed by reaction with a suitable formaldehyde equivalent.



XLIV XLV

The dimeric flavonoids, xanthorrhone and hydroxyxanthorrhone, are, of course, also of biogenetic interest. This is due principally to the fact that they represent the first examples of bisflavonoids having a flavan unit linked to a flavanone unit. There have, however, been many unusual dimeric structural systems found in recent times and the subject has expanded considerably since the isolation of the apigenin dimers ginkgetin[107] (C-C linked), and hinokiflavone[108] (C-O-C linked). A representative sample of the kinds of units and linkages observed in bis-flavonoids is contained in Table 7.

Xanthorrhone and hydroxyxanthorrhone could have been formed by any of three mechanisms: (a) Nucleophilic attack by the readily available flavanone unit on the C-4 carbonium ion produced from an appropriate flavanol by an acid catalysed mechanism. (b) Oxidative coupling of the flavonoid units via a free radical mechanism. (c) Formation of the interflavonyl linkage by interaction of one flavonoid unit with the acetate chain of the second flavonoid unit prior to its cyclisation. (This is a variation of the idea expressed by Wenkert[121]).

There appears to be very little evidence one way or the other, however, (a) is preferred on the basis of

TABLE 7

A-unit*	Linkage A-B**	B-unit*	Reference
naringenin	3-8	naringenin	Scheinmann[104,109]
naringenin	3'-8	naringenin	Miura[110]
apigenin	3'-8	apigenin	Kawano[111]
apigenin	8-8	apigenin	Seshadri[112]
apigenin	4'-0-8	apigenin	Kawano[108]
naringenin	3-8	luteolin	Venkataraman[113]
fisetinidol	4-4	fisetinidol	Russell[114]
catechin	4-8	catechin	Geissman[115]
5,7-dihydroxyflavan	4-8,5-0-7	5,7-dihydroxyflavan	Jurd[116]
catechin	4-8	leucopelargonidin	Ragaswami[117]
dihydroquercetin	4'-0-4	chroman-3,4-diol	Hansel[118]***
3',4',5,7-tetrahydroxyflavan	3-2 (or 4)	3'-hydroxy-leucopelargonidin	Freudenberg[119]
5-deoxycatechin	4-8	mollisacacetin	Drewes[120]

* For comparison purposes the unit is given as the parent phenolic compound.

** The linkage is C-C unless otherwise indicated. In most cases linkages to C-6 or C-8 are equally acceptable.

*** This compound is not, of course, a bis-flavonoid but it is an interesting example of the linkage of two naturally occurring oxygen heterocyclic compounds. Similar compounds such as the flavonolignan, silybin,[122] have also been characterised.

chemical analogy. The flavan-4-ol could have arisen from the flavandiol by a reduction step analogous to that leading to the formation of catechins from flavandiols, or by the reduction of a flavanone.

Mechanism (b) usually involves the one electron oxidation of a phenol by an enzyme followed by radical coupling. Since phenoxy radicals are tautomeric, they can combine in various ways, by C-C, C-O and O-O bonds and by intra- as well as inter- molecular coupling. In the case of the xanthorrhone units, this kind of mechanism probably would have produced an aryl-aryl or aryl-O-aryl linkage and this, of course, is not the case.

With regard to mechanism (c) it would be of interest to examine the apigenin dimers and determine whether generally-labelled apigenin is converted into generally-labelled biflavonyls by feeding experiments.

The isolation of a unique series of naphthopyran derivatives from Xanthorrhoea resin is of particular biogenetic interest and it is worthwhile considering any hypothesis which would account for their formation. Perhaps the most fundamental approach is to consider the function of the assembling enzymes.

Noting a suggestion by Woodward,[123] Whalley[124] has pointed out that since there seems to be an upper limit

of about 18 to the number of carbon atoms a higher plant can organise, flavonoids which already contain 15 carbon atoms probably have little scope for extension. The limiting factors involved are probably the size and shape of the enzymatic area or template available for organisational purposes. With fungi the single long chain of acetyl units seems to be required to bend back on itself only once as in (XLVI). It is interesting to compare the structure of the naturally occurring fatty acids, such as oleic (XLVII), which occur with the cis configuration, with (XLVI).

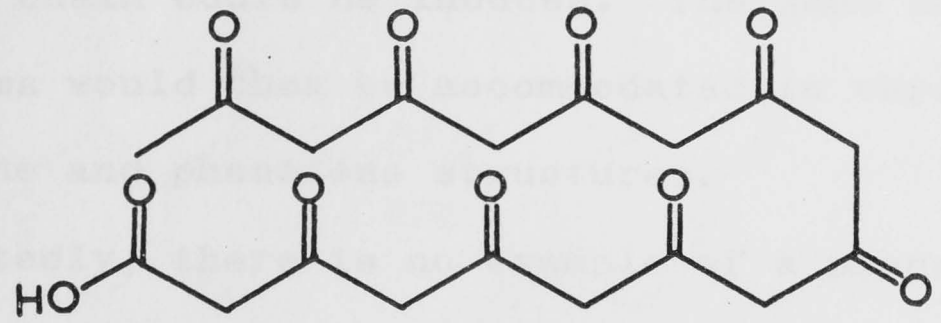
In the higher plants the template is modified so as to receive C_6C_3 acids from the shikimic-prephenic acid system and when this occurs there is little room for anything other than a phloroglucinol nucleus (XLVIII).* In any case the template has probably also lost much of its ability to link acetyl groups together in a larger number.

If, on the other hand, we consider that the template has been modified not so much in function but in shape then it would seem likely that a second bend in the

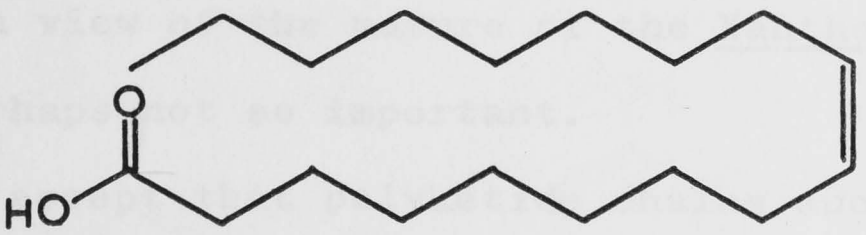
---

*

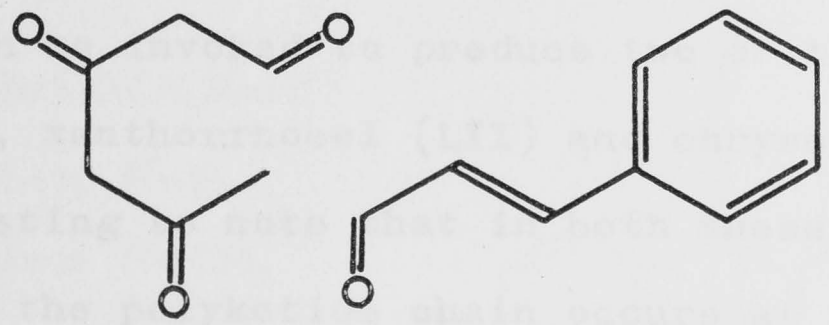
This lends support to the view that dimeric flavonoids are formed by some kind of oxidative coupling.



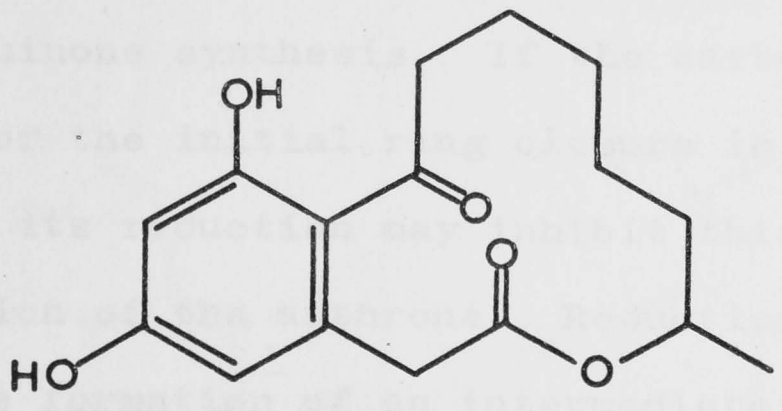
XLVI



XLVII



XLVIII



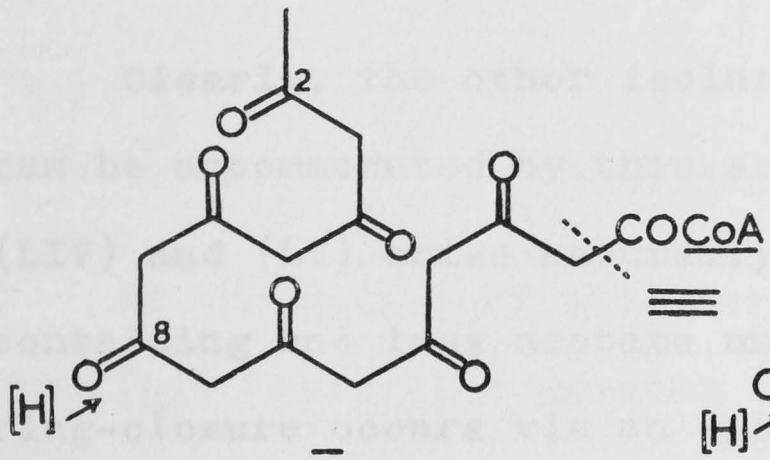
XLIX

polyketide chain could be induced. The same number of carbon atoms would then be accommodated in various phenanthrene and phenalene structures.

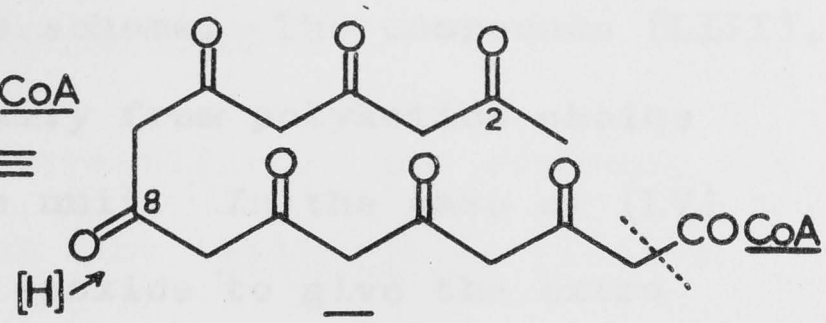
Admittedly, there is no example of a phenanthrene derivative being isolated from a natural source, the nearest example probably being curvularin (XLIX), however, in view of the nature of the Xanthorrhoea plant this is perhaps not so important.

If we accept that polyketide chains such as (L) and (LI) can be assembled (the carboxyl group may occur at either end of the chain) then normal biosynthetic processes can be invoked to produce two of the resin constituents, xanthorrhoeol (LII) and chrysophanic acid. It is interesting to note that in both these examples reduction of the polyketide chain occurs at the same carbon atom (C-8).

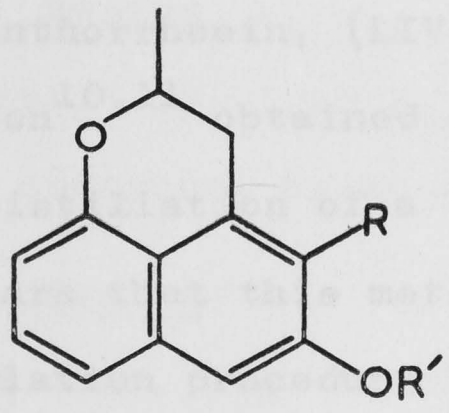
Naphthopyran synthesis appears to be an "off-shoot" of anthraquinone synthesis. If the carbonyl at C-2 is required for the initial ring closure in anthraquinone formation, its reduction may inhibit this and prevent the formation of the anthrone. Reduction at C-2 could lead to the formation of an intermediate such as (LVI) which could undergo ring-closure via proton attack (analogous with diterpene synthesis)to give the naphthopyran system.



L



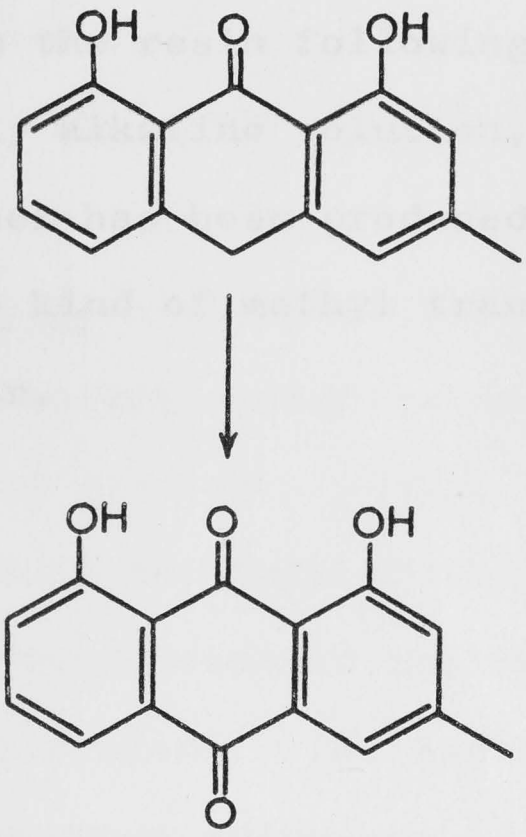
LI



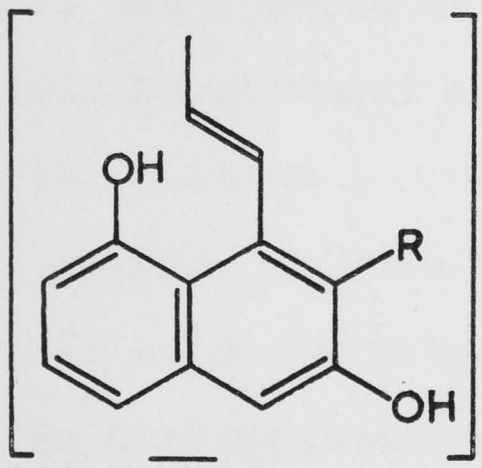
LII ; R=CO·CH$_3$, R'=H

LIII ; R=H , R'=H

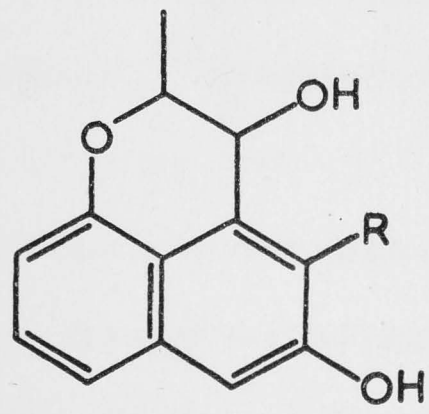
LIV ; R=H , R'=CH$_3$



chrysophanic acid



LVI



LV ; R=H

Clearly, the other isolated naphthopyran derivatives can be accommodated by this scheme. The compounds (LIII), (LIV) and (LV) arise naturally from polyketide chains containing one less acetate unit. In the case of (LV) ring-closure occurs via an epoxide to give the extra hydroxyl group.

Xanthorrhoein, (LIV), has not been isolated since Finlayson[10,11] obtained it from the resin following steam distillation of a strongly alkaline solution, so it appears that this methyl ether has been produced in the isolation procedure by some kind of methyl transfer reaction, possibly from an ester.

6. Chemotaxonomy

At the outset of this investigation the evidence to hand (cf. Table 2) suggested that Xanthorrhoea resins could be divided into two groups.

Class A Resins

Red resins, e.g. from X. australis

Contain compounds based on C_6C_3, $(C_2)_n$ and $C_6C_3(C_2)_3$ units.

Class B Resins

Yellow resins, e.g. from X. hastile

Contain compounds based on C_6C_3 units; either as monomers or polymers.

It was apparent, however, that the bulk of the resins, of both kinds, had not been accounted for and other combinations might well be present. The isolation of chrysophanic acid, an anthraquinone which was apparently not present as such in the original resin but which could be produced as an artefact,[22] tended to support this belief.

The results of this investigation do not provide any further evidence that compounds based on other biogenetic combinations occur in the resins. Nevertheless, they are useful in that they permit some refinements to

be made to the above classifications and also provide a basis for some general observations.

Compounds derived from $C_6C_3(C_2)_3$ units are now known to exist in both classes of resin. In the Class B resins, however, they appear to be present in only relatively small amounts and in addition tend to show a greater degree of substitution in the shikimic acid-derived aromatic ring. The overall variety in the substitution patterns of the flavonoids found in the resins argues either that the plant possesses a large range of synthetic enzymes of different specificities, or else that the same enzymes may be able to deal with different substrates, probably at different rates. The latter is the position which appears to hold for Dahlia variabilis[98] although in the present case the diversity of compounds is even greater.

The differences between the resins can be explained if it is considered that the composition of a resin is a reflection of the normal biosynthetic processes within the plant that is producing it. The ability of Xanthorrhoea plants to link acetate-malonate units together, to link them to C_6C_3 units and to hydroxylate C_6C_3 or $C_6C_3C_6$ units clearly differs from plant to plant, particularly between plants producing different classes

of resin. If the ability to perform these functions is related to the position occupied by the plant in the evolutionary sequence, a position determined either by natural rates of development or by the effects of mutation, it follows that the resins could be more accurately classified on the basis that they were produced by "primitive" or "advanced" species.

The decision on which type of resin is produced by the "advanced" species cannot be made with certainty until the physiological role of the compounds in the plant is determined. Nevertheless, it seems a reasonable postulate that in this case the more "advanced" species will produce a greater diversity of products. In this context, the "advanced" species are those giving Class A resins.

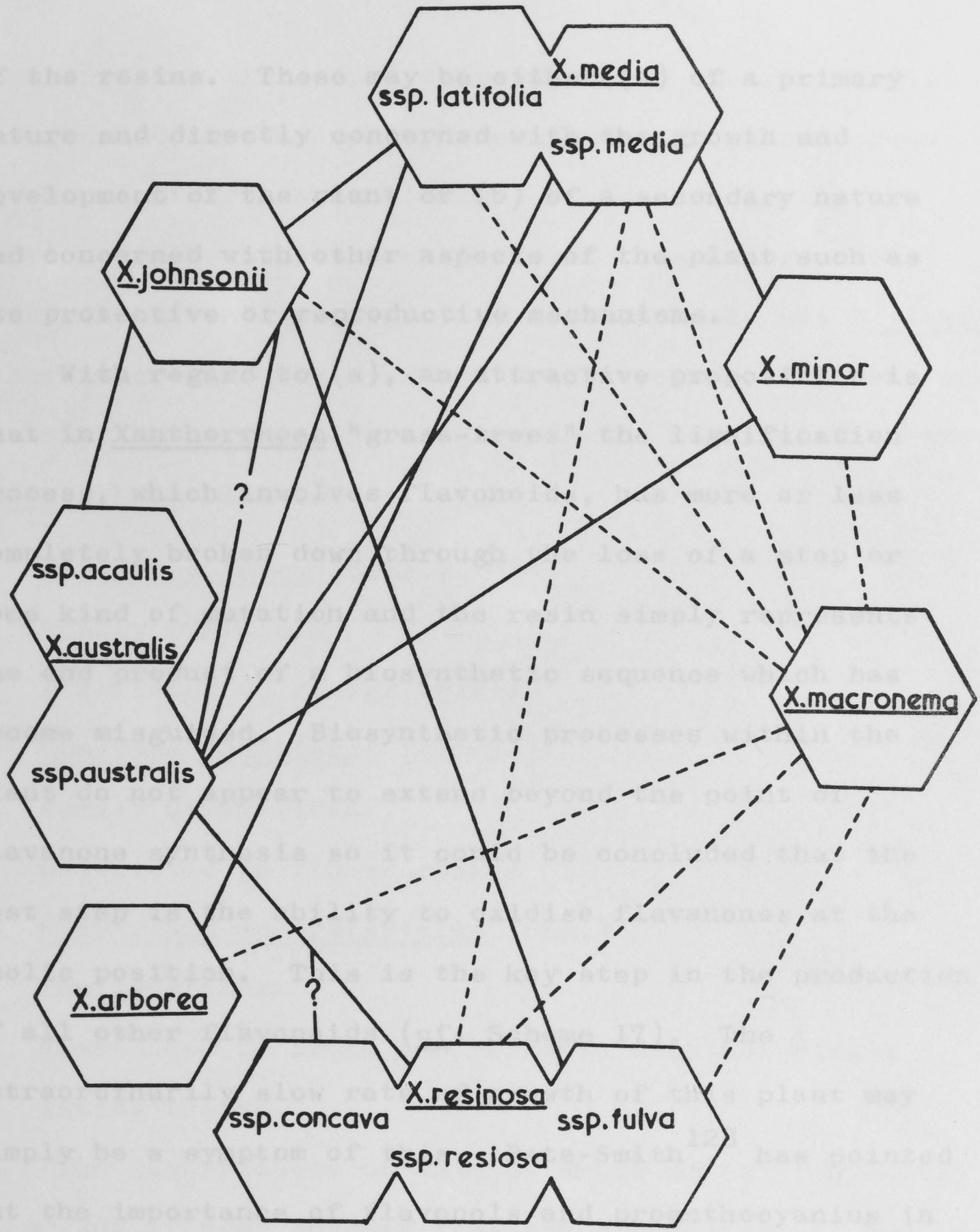
Minor evidence to support this belief comes from an examination of particular constituents of the resins. For example, the compounds derived from $C_6C_3(C_2)_3$ units which are found in the red resins differ from those found in yellow resin in that they contain significant quantities of 5-deoxyflavonoids. Since removal of this hydroxyl group involves an extra reduction step this could be a good indication of an "advanced" species. On the other hand, it could be argued that as the yellow

resins contain flavonoids more highly substituted in the B ring, the extra hydroxylation step, either at the C_6C_3 or $C_6C_3C_6$ stage, also indicates an "advanced" species.

Whether there is any merit in the above approach is certainly debatable. If there are in fact discrete "primitive" and "advanced" species the differences could be reflected in the morphology of the plants.[125] This is apparently not the case as X. hastile, the species from which the yellow resin was obtained, has been recently renamed as simply the sub-species resinosa of X. resinosa which gives a red resin.[127] In her articles on "Xanthorrhoea in Eastern Australia", Lee[126,127] claims that with the exception of X. macronema (a very small species) no species of Xanthorrhoea remains completely discrete. Many putative hybrid derivatives exist. (cf. Fig. 11).

There seems some support for this view in that the author has not been able to find two samples of resin from the "same species", from different localities, which exhibit identical silica chromatograms. It is not possible to evaluate the significance of the minor differences that are observed.

This brings us to an examination of the possible physiological functions which give rise to the production



——— joins taxa having putative hybrid derivatives

------- joins taxa which occur together apparently without hybridism

FIGURE 11

of the resins. These may be either (a) of a primary nature and directly concerned with the growth and development of the plant or (b) of a secondary nature and concerned with other aspects of the plant such as its protective or reproductive mechanisms.

With regard to (a), an attractive proposition is that in Xanthorrhoea "grass-trees" the lignification process, which involves flavonoids, has more or less completely broken down through the loss of a step or some kind of mutation and the resin simply represents the end product of a biosynthetic sequence which has become misguided. Biosynthetic processes within the plant do not appear to extend beyond the point of flavanone synthesis so it could be concluded that the lost step is the ability to oxidise flavanones at the enolic position. This is the key step in the production of all other flavonoids (cf. Scheme 17). The extraordinarily slow rate of growth of this plant may simply be a symptom of this. Bate-Smith[128] has pointed out the importance of flavonols and proanthocyanins in the lignification process and to date these have not been found in the resins.

In so far as (b) is concerned the resins may provide mechanical or antifungal protection. It also seems likely,

however, that the actual composition of the resins will be affected in some way by the physiological responses of the plant to insect attack. It was previously mentioned (cf. p.111) that the most common insect pest found in the inflorescences of xanthorrhoeas was H. latro. Other insect infestations are fairly common, however, and at least three species are known to be restricted to the Xanthorrhoea plant.[65]

1) Hylaletis latro (Zell.)

Family PYRALIDAE

Restricted to flower spikes

2) Comodica acontistes (Meyr.)

Family LYONETIIDAE

Restricted to flower spikes

3) Moerarchis australasiella (Don.)

Family TINEDAE

Restricted to trunk and probably does not attack living tissue.

The significance of this evidence is not completely clear but it does suggest that the specificity of the insects for this plant and the appearance of a unique series of compounds such as the naphthopyrans may be related. Suitable feeding experiments should satisfactorily resolve this point.

Finally, it has been noted that certain phenotypes of Xanthorrhoea often show a strong correlation with soil or other habitat characters. Whether the plants have distinct habitat preferences which cause their location or whether the phenotype is caused by its habitat is a question worthy of further investigation as it may have a direct bearing on the composition of the resin produced.

EXPERIMENTAL

Melting Points

All melting points were measured on a Kofler hot stage and are uncorrected.

Extraction of resin from X. preissii

Plant material, consisting of leaf bases and adhering resin (2600 g.), was extracted with acetone (70 l.) in several portions at room temperature. Solvent was removed on a cyclone evaporator and then on a rotatory evaporator (below 50^{o}) until a black viscous solution (900 ml.; ca. 1100 g.) was obtained. Diethyl ether (10 l.) was added and the mixture was thoroughly stirred and allowed to stand overnight. A small amount of fatty material was deposited. This deposit was removed by filtration and the filtrate concentrated to 2 l.

Bicarbonate-Soluble Fraction (X. preissii)

The ethereal solution of the resin (2 l.) was extracted with a 10 per cent solution of potassium bicarbonate (4 l.) in 800 ml. portions. The aqueous extract was washed with ether (2 l.), acidified with 2N hydrochloric acid, and then extracted with ether (4 l.).

The solution containing the bicarbonate-soluble material was dried over anhydrous magnesium sulphate and evaporated to give a yellow gum. (ca. 4.5 g.).

Sodium Carbonate-Soluble Fraction (X. preissii)

The ethereal solution remaining from the bicarbonate extraction (ca. 2 l.) was extracted with 10 per cent aqueous sodium carbonate solution. (24 portions of 500 ml.). The ethereal solution was then washed with water (2 l.) and the washings added to the carbonate extract. After the aqueous extract had been washed with ether (5 portions of 1600 ml.) it was neutralised with 2N hydrochloric acid and extracted with ether (14 l.) in several portions. The ethereal solution was washed with water (14 l.), dried over anhydrous magnesium sulphate and evaporated to give a dark brown viscous tar (ca. 200 g.).

Carbonate-Insoluble Fraction (X. preissii)

The ethereal solution containing the carbonate-insoluble material was washed with water (2 l. in 4 portions), dried over anhydrous magnesium sulphate and evaporated under reduced pressure and yielded a dark brown tar (ca. 700 g.).

Chromatography of Carbonate-Soluble Fraction (X. preissii)

The viscous tar comprising the carbonate-soluble fraction was extracted with warm benzene (250 ml.) for 13 hr. The solvent was decanted and on evaporation under reduced pressure yielded a sticky brown gum (70 g.). A sample of this material (6.5 g.) was taken up in benzene and chromatographed on a silicic acid column (250 g. in benzene).

Chromatography of Sodium Carbonate-Insoluble Fraction (X.preissii)

A sample of the carbonate-insoluble fraction (ca. 25g.) was taken up in methanol (100 ml.) and precipitated solid removed by filtration. The solution was evaporated under reduced pressure and portion of the residue (10 g.) was used to prepare a slurry with chromatographic-grade silicic acid and benzene. This slurry was added to the top of a silicic acid column (1 Kg. in benzene) which was then eluted with benzene, benzene-chloroform solutions (per cent chloroform : 1, 2, 5, 10, 25, 50) and chloroform. 20 ml. fractions (420) were collected, representing a total recovery of 6.65 g. Fractions were combined on the basis of physical appearance, u.v.

absorption spectra (in ethanol) and weight (from a weight of fraction *vs.* fraction number curve).

Preparation of Phloroacetophenone

Phloroacetophenone was prepared from phloroglucinol and acetonitrile according to the method of Venkataraman *et al.*[131] It was obtained in 80 per cent yield as pale yellow crystals, m.p. 217-219° (lit.,[131] 217-219°).

Preparation of 2,4-Dimethoxy-6-hydroxyacetophenone

Methylation of phloroacetophenone with dimethylsulphate in acetone in the presence of potassium carbonate by the usual method gave a 65 per cent yield of the dimethyl ether. It was purified by chromatography on silica and recrystallised from ethanol as colourless plates, m.p. 81° (lit.,[129] 82-83°). The i.r. spectrum (CCl_4) showed strong absorption at 1620 cm^{-1} (C = O), 1265 cm^{-1}, 1210 cm^{-1} (C-O), 1160 cm^{-1}, 1120 cm^{-1} (OMe), weak absorption at 2850 cm^{-1} (Ar-O-Me) and normal aromatic absorption bands.

Preparation of 2',4'-Dimethoxy-6'-hydroxychalcone

2',4'-Dimethoxy-6'-hydroxychalcone was prepared by the base catalysed condensation of 2,4-dimethoxy-6-hydroxyacetophenone and benzaldehyde according to the

method of Kohler and Chadwell.[132] The chalcone was obtained as orange crystals, m.p. 90-91° (lit.,[129] 91-92°), in 72 per cent yield.

Preparation of 5,7-Dimethoxyflavanone

2'-Hydroxy-4,6-dimethoxychalcone was cyclised to the corresponding flavanone by the method of Eneback and Gripenberg.[133] Colourless needles, m.p. 143-144° (lit.,[68] 144°), from methanol, were obtained in 35 per cent yield. (m.p. 169° also claimed[130,28] appears to be incorrect.) $\lambda_{max.}$ (ethanol) 283.5 mμ(4.20), 310 mμ(shoulder). No shift of $\lambda_{max.}$ on addition of alkali. C = O absorption frequency (nujol) 1675 cm^{-1}.

Preparation of 5,7-Dimethoxyflavan-4-ol

5,7-Dimethoxyflavanone (30 mg.) was dissolved in methanol (10 ml.) and sodium borohydride (8 mg.) added. The solution was allowed to stand ½ hr. and then solvent was removed, under reduced pressure, until the volume was approximately 2 ml. Water (15 ml.) was added and this was followed by the addition of solid carbon dioxide until the solution had been neutralised. The solution was then extracted with ether (30 ml.) and this extract was washed with water, dried over anhydrous magnesium sulphate and evaporated. A quantitative yield of a

colourless oil was obtained. This oil, which became virtually solid on drying under reduced pressure, showed only benzenoid absorption in the u.v. spectrum (in ethanol) and no carbonyl absorption in the i.r. spectrum. Hydroxyl absorption appeared at 3550 cm^{-1}. The p.m.r. spectrum of this compound has been previously discussed. (See p.64).

Preparation of 5,7-Dimethoxy-4(C-phloroglucinyl)flavan

To phloroglucinol (620 mg.) in aqueous dioxan (50 per cent, 20 ml.) containing 0.5 N hydrochloric acid (5 ml.) was added 5,7-dimethoxyflavan-4-ol (283 mg.) in aqueous dioxan (3:7, 40 ml.) dropwise over 1.5 hr. with stirring. After 2 hr. the solution was reduced to 20 ml. on a rotary evaporator and the mixture was extracted with ether. The extract was washed with aqueous sodium bicarbonate and with water and then evaporated to yield an amorphous solid. Thin-layer chromatography on silica gel (H) in chloroform-ether (50 per cent) showed essentially four components: unchanged flavanol (Rf 0.58), phloroglucinol (Rf 0.30), dimeric flavan, m/e 536 (Rf 0.71), and the required phloroglucinylflavan (Rf 0.41). Separation on a silicic acid column with chloroform gave the latter substance (95 mg.), m.p. 120-122° raised to

m.p. 128-129° by crystallisation from benzene. The structure was fully supported by p.m.r. and mass spectra (See pp.66, 69).

Synthesis of Xanthorrhone

5,7-Dihydroxyflavanone (257 mg.) was dissolved in aqueous dioxan (50 per cent, 25 ml.) containing 0.5 N hydrochloric acid (2 ml.). To it was added dropwise, 5,7-dimethoxyflavan-4-ol (260 mg.) in aqueous dioxan (3:7, 35 ml.) during 1.5 hr. Water (50 ml.) was added after 20 hr. and the solution was extracted several times with ether. The extract was washed with sodium bicarbonate solution and water, and evaporated. Examination by t.l.c. on silica gel (H) in chloroform-ether (1:1) showed a small proportion of material (Rf 0.35) identical in Rf to xanthorrhone. This material was isolated by column chromatography on silica and finally by preparative t.l.c. on silica gel (H). The racemic xanthorrhone (1.5 mg.) had m.p. 190-192° with a phase-change at 165-170°, identical with natural xanthorrhone and undepressed by admixture with it. The ultra-violet spectrum and the changes induced by alkali and by aluminium chloride were in agreement with those of natural xanthorrhone. Xanthorrhone (naturally

occurring): $\lambda_{max.}$ (EtOH), 297 mμ, 336 mμ (shoulder). Bathochromic shifts of 40 mμ and 21 mμ were observed on the addition of alkali and aluminium chloride respectively. Xanthorrhone (synthetic): $\lambda_{max.}$ (EtOH), 297 mμ, 340 mμ (shoulder). Bathochromic shifts of 40 mμ and 20 mμ were observed on the addition of alkali and aluminium chloride. Attempts to repeat this synthesis failed.

Preparation of Palladium on Carbon Catalyst

Palladium on carbon catalyst (10 g.) was prepared by the hydrogenation of an aqueous mixture of palladium chloride, sodium acetate and norite according to the method of Hartung.[134]

Conversion of Chrysin into Pinocembrin

Reduction of the flavone by the method of Massicot et al.[135] using palladium on carbon in tetralin gave a 25 per cent yield of 5,7-dihydroxyflavanone, m.p. 200° (from alcohol)(lit.,[41] 200°). The u.v. absorption spectrum of this compound showed $\lambda_{max.}$ (ethanol) at 290 mμ (4.20) and 328 mμ (shoulder), while the i.r. spectrum (nujol) showed carbonyl absorption at 1638 cm^{-1}.

The physical and spectral properties of synthetic 5, 7-dihydroxyflavanone were in complete agreement with those

of the naturally occurring material obtained from the carbonate-soluble fraction of X. preissii. (PC18).

Hydrolysis of 2-Phenylethyl phenylacetate: (F14)

A sample of fraction F14 (90 mg.) was dissolved in warm ethanolic sodium carbonate (10 per cent)(20 ml.) and the solution was allowed to cool to room temperature and stand for 12 hr. Most of the solvent was then removed under reduced pressure, water (25 ml.) added and the solution extracted with ether. This extract was examined by gas chromatography (Carbowax 20M column) and found to contain material with a retention time identical to that of 2-phenylethanol.

Acidification of the carbonate solution with 2N hydrochloric acid and extraction with ether gave the acidic product of the hydrolysis. The ethereal solution was washed with water, dried over magnesium sulphate and allowed to evaporate to dryness at room temperature. Colourless plates of phenylacetic acid m.p. 73-75° (lit.,[68] 76°) were obtained.

Synthesis of 2-Phenylethyl phenylacetate

2-Phenylethanol (12 g.) and phenylacetic acid (15 g.) were heated under reflux [redacted] with conc. sulphuric

acid (1 ml.) for 3 hr. Water (60 ml.) was then added and the mixture was extracted with ether. The ethereal solution was washed with sodium bicarbonate solution and water, dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The residual oil was distilled under reduced pressure (0.3 mm.) and the fraction b.p. 150-160° (23g., 90 per cent) collected. This material showed carbonyl absorption at 1738 cm^{-1}. The ester was examined by gas chromatography (Silicone Gum rubber E-301 column at 150°) and found to have the same retention time (12.5 min.) as fraction F14.

Conversion of 4',5-dihydroxy-7-methoxyflavanone (sakuranetin) into 4',5-dihydroxy-7-methoxyflavone (genkwanin).

The dehydrogenation of sakuranetin was carried out with iodine and acetic acid in the presence of freshly fused potassium acetate according to the method of Goel and Seshadri.[136] The flavone, obtained in 90 per cent yield, was recrystallised from methanol as pale yellow needles, m.p. 286-287° (lit.,[68] 286°). $\lambda_{max.}$ (ethanol): 268 mμ (4.22), 336 mμ (4.23). The physical and spectral properties agreed with those reported by Asen and Jurd[137] for genkwanin.

Methylation of Phenols

Throughout this investigation all methylation procedures involved the use of either diazomethane or dimethylsulphate.

Ethereal solutions of diazomethane (0.35g./10 ml.) were prepared and used in accordance with the methods outlined by Vogel.[138]

Methylations using dimethylsulphate were carried out in acetone solution in the presence of potassium carbonate.[139]

In all cases, fully methylated compounds were purified by column chromatography on alumina and partially methylated compounds (i.e. flavonoids containing unmethylated 5-hydroxy groups) were obtained by preparative t.l.c. on silica gel.

Methylation of 4',5-dihydroxy-7-methoxyflavone (genkwanin) and 5,7-dihydroxy-4'-methoxyflavone (acacetin)

Confirmation of the structure of sakuranetin (4',5-dihydroxy-7-methoxyflavanone) was obtained by methylating its flavone derivative and comparing the product with methylated acacetin. Both methylations were carried out under identical conditions using an ethereal solution of diazomethane.[138] (Note: The flavones genkwanin and

acacetin have a comparatively low solubility in ether, ca. 1 mg./ml.). The product from the methylations, 4',7-dimethoxy-5-hydroxyflavone, was obtained as pale yellow needles, m.p. 169-170° (from ethanol) (lit.,[68] 171-172°). Rf = 0.3 (Silica gel H; chloroform).

Extraction of Resin from Xanthorrhoea australis

A plant specimen was collected from Mt. McDonald in the A.C.T. The community from which it was obtained comprised several hundred members and existed near the top of the mountain in poor, acidic soil. Although the area had been subject to bushfires, the particular specimen collected showed signs of charring on only half of its 5' length. The plant also carried a yellow inflorescence.

The specimen was divided into three portions:

(a) Dried out leaf bases with brittle resin

(b) Sticky resin

(c) Inflorescence material

(a) Brittle Resin

The plant material, previously stripped of excessive dry leaf, was broken apart and the central stem removed. The remaining material consisting of dry leaf bases with

adhering resin (3 kg.) was broken into small pieces and placed in an earthenware pot together with petroleum ether b.p. 60-80° (18 l.). The mixture was allowed to stand at room temperature, with occasional agitation, for 7 days. The petrol extract was then removed (decantation/filtration) and evaporated to dryness by means of cyclone and rotatory evaporators. Fatty material was removed by precipitation and filtration from a cold ether-methanol solution (50 per cent; 100 ml.) and the filtrate evaporated to dryness. The residue (5.7 g.) was taken up in ether (100 ml.) and divided into a bicarbonate-soluble fraction (0.1 g.), a carbonate-soluble fraction (1.1 g.), an alkali-soluble fraction (2.9 g.), and a neutral fraction (1.3 g.) in the usual manner.

The plant material which remained after the extraction with petroleum ether was dried in the atmosphere at room temperature and replaced in an earthenware pot. It was extracted at room temperature with acetone (54 l. in 4 portions) and the extract was concentrated under reduced pressure to a black viscous tar (266 g.). This material was divided into petrol-soluble (a), carbon tetrachloride-soluble (b), benzene-soluble (c), and benzene-insoluble (d) fractions by the following procedure:

(a) To the acetone extract was added petroleum ether b.p. 60-80° (400 ml.). The mixture was kept at 45-50° for 4 hr. with occasional stirring, cooled to room temperature and the solvent decanted. The petrol solution was evaporated to dryness under reduced pressure and the residue (6.5 g.) taken up in methanol (100 ml.). Insoluble fatty material was removed by filtration and the filtrate evaporated to give a yellow oil (3.3 g.).

(b) The resinous material not extracted in (a) was treated with carbon tetrachloride (500 ml., 400 ml., 300 ml.; 35°, 1 hr.). In each case the mixture was cooled and the solvent decanted. The extracts were then combined and evaporated under reduced pressure. A dark brown viscous tar (175 g.) was obtained.

(c) Material not extracted in (b) was treated with benzene. (400 ml., 300 ml., 300 ml.; 45-50°, 3 hr.). The combined extracts were evaporated to give a brown tar (40 g.).

(d) The resinous material not extracted in (c) was dried under reduced pressure (ca. 47 g.).

It was not possible to completely dry any of the extracts. Even under comparatively poor drying conditions (ca. 10 mm. pressure) the resin swelled and filled the containing vessel.

X. australis-acetone extract/carbon tetrachloride-soluble fraction.

The extract from (b) above (175 g.) was taken up in methanol (1200 ml.) and precipitated fatty material (9 g.) removed by filtration. The methanol was then evaporated under reduced pressure and ether (1 l.) was added. The ethereal solution was washed with water (1 l. in 2 portions) and then extracted with 5 per cent sodium bicarbonate solution (1 l. in 4 portions) and 5 per cent sodium carbonate solution (4 l. in 20 portions). In the case of the carbonate extraction it was necessary to add a small quantity of finely powdered magnesium sulphate to define the ether-water interface. The extracts were neutralised and worked up in the normal manner. The bicarbonate-soluble fraction (0.57 g.), the carbonate-soluble fraction (36.9 g.) and the carbonate-insoluble fraction (85 g.) were examined by t.l.c. on silica gel.

Ozonolysis of Xanthorrhoeol

Xanthorrhoeol (50 mg.) was dissolved in chloroform-methanol (50 per cent; 15 ml.) and a stream of ozone (25 ml./min.) passed through the solution, at room temperature, for 2 hr. During this period the deep yellow solution became colourless. A solution of

hydrogen peroxide-formic acid (100 per cent HCOOH/30 per cent H_2O_2, 1:1; 5 ml.) was added and the solution allowed to stand 18 hr. The reaction mixture was then diluted with an equal volume of H_2O and exhaustively extracted with chloroform. This extract was washed with a saturated solution of sodium sulphite and then with water. It was dried over magnesium sulphate and evaporated. The residue (10 mg.) was examined by t.l.c. (silica gel H; water-saturated-ether/formic acid, 7:1) and found to contain material with Rf = 0.7 which corresponded with authentic β-hydroxybutyric acid.

Preparation of copper complex of 5,7-dihydroxyflavanone

To a solution of 5,7-dihydroxyflavanone (3 mg.) in methanol (2 ml.) was added a saturated solution of cupric acetate in methanol (10 ml.). Immediately a colloidal precipitate was formed. The solution was warmed under gentle reflux for 1 hr., cooled, and the solid collected by centrifugation. The complex was washed with small quantities of methanol until the washings were colourless. The dried complex was obtained as a green amorphous solid, m.p. 315-322°d. Although the solubility of the material in chloroform was low, a u.v. absorption spectrum was obtainable. It showed $\lambda_{max.}$ 323 mμ.

On the addition of a drop of conc. hydrochloric acid to the chloroform solution the complex was destroyed and the spectrum reverted to that of 5,7-dihydroxyflavanone. ($\lambda_{max.}$ 290 mμ, 327 mμ (shoulder)). The i.r. absorption spectrum (nujol) indicated that the carbonyl absorption of the flavanone had been lowered 25 cm^{-1} to 1615 cm^{-1} by complex formation.

Separation of Resin Components on Basis of Complex Formation

Fraction (d) above (brittle resin/acetone extract/benzene-insoluble)(47 g.) was dissolved in methanol (250 ml.) and the solution heated under gentle reflux. Water (250 ml.) was added to the hot solution in small portions (ca. 70 ml.), and the solution was allowed to cool to room temperature. Precipitated tar was removed by decantation and filtration through a cotton wool plug and the resulting solution evaporated to ca. 700 ml. under reduced pressure. The aqueous mixture obtained in this manner was extracted with ethyl acetate (1 l.) and this extract was evaporated to dryness.

A sample of the ethyl acetate-soluble material (7.2 g.) was taken up in methanol (30 ml.) and a hot saturated solution of cupric acetate in methanol (150 ml.)

added. The solution was brought to gentle reflux and allowed to cool to room temperature. Deposited solid was removed by centrifugation and the methanol solution filtered through a bed of hyflo-super cell and evaporated to dryness.

The non-complexed material, mixed with methanol-soluble complex and excess cupric acetate, (5.1 g.) was taken up in ethyl acetate (50 ml.). Water (50 ml.) and a few drops of hydrochloric acid were added to decompose any complex present and the mixture was vigorously shaken. The aqueous layer was removed and the ethyl acetate solution washed with several portions of water, dried over magnesium sulphate and evaporated. The residue was extracted with ether and then divided up into a bicarbonate-soluble fraction (0.02 g.) a carbonate-soluble fraction (1.59 g.), an alkali-soluble fraction (0.56 g.), and a neutral fraction (0.04 g.) by the usual methods.

(b) Sticky Resin

The young fleshy leaf bases (200 g.) were broken apart and the thin film of yellow sticky material adhering to them removed by extraction at room temperature with (i) petroleum ether, b.p. 40-60° (750 ml. for 5 days; 750 ml. for 3 days) and (ii) acetone (200 ml., 15 hr.).

Fatty material was removed from the petrol extract by precipitation from cold methanol (50 ml.) and the extract was divided into bicarbonate-soluble, carbonate-soluble and carbonate-insoluble fractions in the usual way. The bicarbonate-soluble fraction was insignificant, the carbonate-soluble fraction amounted to 278 mg. and the carbonate-insoluble fraction to 803 mg.

The acetone extract was divided up in a similar manner into a bicarbonate-soluble fraction (20 mg.), a carbonate-soluble fraction (1.08 g.) and a carbonate-insoluble fraction (6.1 g.). The latter was taken up in ether (50 ml.) and extracted with 6 portions of ice-cold 5% sodium hydroxide solution (10 ml.). The extract was neutralised, extracted with ether etc. in the usual manner and evaporated to give a brown viscous oil (5.8 g.).

(c) Inflorescence Material

Inflorescence material (scape and spike)(20 Kg.) was collected in the flowering season (mid-November). The material was chopped into thin slices and extracted with ethanol (18 gals. in 3 portions). The dry debris after the extraction amounted to 2 Kg.

The ethanol extract was concentrated to 8 l.(cyclone evaporator) and half of this material (a suspension) was

further concentrated to $2\frac{1}{2}$ l. and continuously extracted with ether. Evaporation of the dried ether solution gave a strongly smelling dark brown mobile oil (65 g.). This extract was taken up in methanol (1 l.), precipitated material removed by filtration and the solvent then removed to give a brown oil (50 g.). Ether (500 ml.) was added and the ethereal solution was extracted with 10% aqueous potassium bicarbonate solution (2 l.), 10% aqueous sodium carbonate (1200 ml.) and ice-cold 10% aqueous sodium hydroxide solution (1 l.). Normal work-up procedures gave the bicarbonate-soluble fraction (1 g.), carbonate-soluble fraction (1.5 g.) and alkali-soluble fraction (250 mg.).

It is noteworthy that only a small proportion of the crude extract was found to be ether soluble. Emulsions were very easily formed and difficult to destroy, and in attempts to maintain the true identity of the various fractions it became necessary to discard considerable amounts of material.

Fractionation of Resin from X. hastile

Xanthorrhoea hastile resin (2 Kg.; previously extracted from the plant material) was dissolved in acetone (10 l.) and the volume reduced to ca. 2 l.

This concentrate was slowly added, with stirring, to ether (6 l.). The precipitated material was filtered off and the filtrate concentrated to 1 l. Half of this concentrate was added to ether (4 l.) and further precipitated material removed. The ethereal solution was extracted with 10 per cent sodium carbonate solution (10 l. in 10 portions) and the extract back-washed with ether (1 l.) in the usual way. The carbonate-insoluble fraction was dried over anhydrous magnesium sulphate and the solvent removed to give a dark brown viscous tar (670 g.). The carbonate-soluble fraction was neutralised with 2N hydrochloric acid and extracted with ether (5 l. in 5 portions). The ether solution (conveniently divided into 2 aliquots) was washed with water and concentrated to 2 l. This solution was extracted with 10 per cent potassium bicarbonate solution (1 l. in 4 portions), washed with water, dried over anhydrous magnesium sulphate and evaporated to give a brown amorphous low-melting solid (ca. 100g.). The bicarbonate extract was worked up in the normal way to give a yellow amorphous solid (40 g.).

The carbonate-insoluble fraction was taken up in ether and extracted with ice-cold 5% sodium hydroxide solution (2 l.). An alkali-soluble fraction (ca. 640g.),

and a neutral fraction (29 g.) were obtained from the subsequent work-up.

Reductive Cleavage of Flavonoids with Sodium-mercury Amalgam.

The method of Hurst and Harborne[77] was used. The best results were obtained using 5 per cent sodium-mercury amalgam (6 g.), a 10 mg. sample and heating the reaction mixture 1 hr. Silica gel HF_{254} (fluorescent) was found to be useful in the detection of the compounds in the two-dimensional chromatogram.

Hydrolysis of Hesperidin

The method of Arthur, Hui and Ma[142] gives an almost quantitative yield of hesperetin in a smooth hydrolysis with ethylene glycol and sulphuric acid. The product was obtained as pale yellow needles from aqueous ethanol, m.p. 226-227° (lit.,[142] 224-226°).

Chromatography of Resin Components and their Derivatives

(a) Paper Chromatography:

Paper chromatography was not extensively used in the investigation, however, some useful qualitative results were obtained with "Whatman No. 1" paper and 50 per cent aqueous methanol as the developing solution.

(b) Column Chromatography:

Several grades of silicic acid were tried. The best results were obtained with "Mallincrodt", 100-mesh, or "Sigma" SIL-LC, 325-mesh, eluted with benzene or chloroform solutions. "Merck" neutral alumina (activity III) and "Merck" or "Ajax" silica gel, 100-200 mesh, were also successfully used.

(c) Thin-layer Chromatography:

For most qualitative and quantitative purposes, "Merck" silica gel, G or H (with or without fluorescent additive) was used. The usual developing solutions were benzene, chloroform (developed several times if necessary), chloroform-ether, and benzene-ethyl acetate. Other solvent systems, including toluene-ethyl formate-formic acid (5:4:1) and benzene-dioxan-acetic acid (90:25:4), were useful for separating aromatic carboxylic acids.

Flavonoids were usually differentiated by their colour (visible, u.v., u.v. in presence of NH_3 vapour) or by the colour they developed on being sprayed with ferric chloride solutions or with conc. sulphuric acid. More specific reagents, e.g. antimony pentachloride in carbon tetrachloride for distinguishing flavanones from chalcones, were also used. (cf. Venkataraman[56]).

Thin-layer chromatography on cellulose was not practicable due to excessive "streaking", polyamide layers were useful only for qualitative work, and t.l.c. on alumina had limited application.

Useful information on the separation of phenols is contained in handbooks by Randerath[140] and Stahl[141].

(d) Gas Chromatography

Qualitative gas chromatography was carried out using a Perkin Elmer PE881 gas chromatograph.

Spectroscopy

(a) Mass Spectroscopy

Mass Spectra were measured on a G.E.C.-A.E.I. MS902 double focusing mass spectrometer at 15eV or 70eV. Source temperatures were usually 100-200°.

(b) Infrared Spectroscopy

Measurements were made on a Perkin-Elmer 257 grating infrared spectrophotometer.

(c) Ultraviolet Spectroscopy

Measurements were made on a Unicam S.P.800 ultraviolet spectrophotometer.

(d) Nuclear Magnetic Resonance Spectroscopy

P.m.r. spectra were recorded on a Varian Associates HA 100 N.M.R. Spectrometer. Tetramethylsilane was used as the internal reference and all spectra were measured at room temperature.

BIBLIOGRAPHY

1. A. Lichenstein, Crells Journal, 2, 242 (1799).

2. (a) J. Schrader, Trommsdorff's Journal, 5, 96.

   (b) F.W. Johnson, Annalen, 44, 330 (1842).

   (c) C. Widmann, Buchner's Reportorium, 22, 198 (1825).

   (d) Trommsdorf Gmelin's Handbuch d. Chim., 2, 618 (1826).

   (e) J.W. Johnston, Phil. Trans. Roy. Soc. Lond., 129, 28 (1839).

   (f) J. Stenhouse, Proc. Chem. Soc., 3, 10 (1845); Pharm. J., 6, 88 (1846-7); Annalen, 57, 84 (1846).

   (g) J. Periera, "Elements of Materia Medica and Therapeutics", 2 (Part 1), 1099 (1850).

   (h) P.L. Simmonds, Roy. Soc. Arts. J., 4, 18 (1855); Pharm. J., 8, 78 (1866).

   (i) C.W. Ligar, Roy. Soc. Victoria Trans., 7, 145 (1866).

   (j) J.M. Maisch, Pharm. J., 11, 1005 (1880); Amer. J. Pharm., 53 (1881).

   (k) E.J. Mills and J. Muter, J. Soc. Chem. Ind., 4, 96 (1885).

3. M. Bamberger, Monatsh., 11, 84 (1890); 14, 333 (1893).

4. A. Tschirch and K. Hildebrand, Archiv. d. Pharm., 234, 698.

5. (a) H. Rowley, J. Soc. Chem. Ind., 35, 680 (1916). [Chem.Abs., 11, 1295 (1917)]

(b) J.L. Strevens, Chem. Eng. Mining Rev., 11, 80, (1918). [Chem.Abs., 13, 3024 (1919)]

(c) Anon., Perf. Essent. Oil Rec., 11, 143 (1920). [Chem.Abs., 14, 2393 (1920)]

(d) E. Mackinnon, Sci.Ind. (Aust.), 2, 277 (1920). [Chem.Abs., 14, 2864 (1920)]

(e) H.J. Pooley and J.L. Strevens, Brit. Pat., 160,080 (1920). [Chem.Abs., 15, 2362 (1921)]

(f) F.W. Steel, Chem. Eng. Mining Rev., 24, 362 (1932). [Chem.Abs., 26, 5410 (1932)]

(g) H.T. Cole, Chem. Eng. Mining Rev., 25, 92 (1932). [Chem.Abs., 27, 4426 (1933)]

6. Anon., Bull. Imp. Inst., 18, 155 (1920). [Chem.Abs., 15, 1407 (1921)]

7. E.A. Mann, J. Soc. Chem. Ind., 25, 1076 (1906); West. Australian Dept. Ag. J., 14, 162 (1906).

8. H. Rowley, J. Soc. Chem. Ind., 35, 290 (1916).

9. G. Lightfoot, Aust. Inst. Sci. Ind. Bull., No.20, 104 (1921). [Chem.Abs., 15, 2148 (1921)]

10. E.H. Rennie, W.T. Cooke and H.H. Finlayson, J. Chem. Soc., 117, 338 (1920).

11. H.H. Finlayson, J. Chem. Soc., 2763 (1926).

12. A.J. Birch and P. Hextall, Aust. J. Chem., 8, 263 (1955).

13. A.J. Birch and M. Salahud-Din, Tetrahedron Letters, 2211 (1964).

14. (a) A.J. Birch, M. Salahud-Din and D.C.C. Smith, Tetrahedron Letters, 1623 (1964).

(b) A.J. Birch, M. Salahud-Din and D.C.C. Smith, J. Chem. Soc. (C), 523 (1966).

15. Council for Scientific and Industrial Research, Australia, Bull. Imp. Inst., 42, 74 (1944). [Chem.Abs., 38, 6579 (1944)]

16. A.J. Ryan, B. Sc. (Hons.) Thesis, University of Sydney, 1954.

17. P.J. Hextall, M.Sc. Thesis, University of Sydney, 1955.

18. B.R. McReavie, B.Sc. (Hons.) Thesis, University of Sydney, 1955.

19. H.T. Cooke, Chem. Eng. Mining Rev., 25, 92 (1932). [Chem.Abs., 27, 1527 (1933)]

20. L.H. Briggs and R.H. Locker, J. Chem. Soc., 3136 (1951).

21. (a) T.H. Simpson and L. Garden, J. Chem. Soc., 4638 (1952).

(b) B.L. Shaw and T.H. Simpson, J. Chem. Soc., 5027 (1952).

22. H. Duewell, J. Chem. Soc., 2562 (1954).

23. H. Duewell, Aust. J. Chem., 18, 575 (1965).

24. O. Goncalves de Lima, W. Keller-Schierlein and V. Prelog, Helv., 41, 1386 (1958).

25. J. Comin, O. Goncalves de Lima, H.N. Grant, L.M. Jackman, W. Keller-Schierlein and V. Prelog., Helv., 46, 409 (1963).

26. H. Duewell and T.J. Haig, J. Chem. Soc. (C), 169 (1968).

27. H. Duewell, Aust. J. Chem., 21, 1679 (1968).

28. M. Salahud-Din, Ph.D. Thesis, University of Manchester, 1966.

29. A.J. Birch and M. Salahud-Din, Tetrahedron Letters, 2211 (1964).

30. A. Gaudemer and E. Lederer, Compt. rend., 259, 4167 (1964).

31. (a) K. Kurosawa, W.D. Ollis, B.T. Redman, I.O. Sutherland, A. Braga de Olivera, O.R. Gottlieb and H. Magalhaes Alves, Chem. Comm., 1263 (1968).

(b) K. Kurosawa, W.D. Ollis, B.T. Redman, I.O. Sutherland, O.R. Gottlieb and H. Magalhaes Alves, ibid., 1265 (1968).

32. H. Duewell, private communication.

33. G.H. Mansfield, T. Swain and C.G. Nordstrom, Nature, 172, 23 (1953).

34. (a) J.B. Harborne, Chem. and Ind., 1142 (1954).

(b) T. Swain, Chem. and Ind., 1480 (1954).

35. L. Jurd and R.M. Horowitz, J. Org. Chem., 22, 1618 (1957).

36. J. Shinoda, J. Pharm. Soc. Japan, 48, 214 (1928).

37. (a) C.S. Barnes and J.L. Occolowitz, Aust. J. Chem., 17, 975 (1964).

(b) A. Pelter, P. Stainton and M. Barber, J. Heterocyclic Chem., 2, 262 (1965).

38. I. Fleming and D.H. Williams, "Spectroscopic Methods in Organic Chemistry", McGraw-Hill (London) 1966, p.55.

39. E.A. Braude and F.C. Nachod, "Determination of Organic Structures by Physical Methods", Academic Press, 1955, p.85.

40. (a) R.S. Cahn and C.K. Ingold, J. Chem. Soc., 612 (1951).

(b) R.S. Cahn, C.K. Ingold and V. Prelog, Experentia, 12, 81 (1956).

41. F.M. Dean, "Naturally Occurring Oxygen Ring Compounds", Butterworths, London, 1963, p.357.

42. S. Meyerson, H. Drews and E.K. Fields, J. Amer. Chem. Soc., 86, 4964 (1964).

43. T.S. Wheeler, Record Chem. Prog., 18, 133(1957).

44. S.E. Drewes, D.G. Roux, H.M. Saayman, J. Feeney and S.H. Eggers, Chem. Comm., 370 (1966).

45. J.W. Clark-Lewis, Revs. Pure and Applied Chem., 12, 96 (1962).

46. H.D. Gibbs, J. Biol. Chem., 72, 649 (1927).

47. B.J. Bolger, A. Hirwe, K.G. Marathe, E.M. Philbin, M.A. Vickars and C.P. Lillya, Tetrahedron, 22, 621 (1966).

48. T.A. Geissman and H.F.K. Dittmar, Phytochem., 4, 359 (1965).

49. T.A. Geissman and N.N. Yoshimura, Tetrahedron Letters, 2669 (1966).

50. L. Jurd and R. Lundin, Tetrahedron, 24, 2653 (1968).

51. C.P. Lillya, S.E. Drewes and D.G. Roux, Chem. and Ind., 783 (1963).

52. A.J. Birch and M. Slaytor, Chem. and Ind., 1524 (1956).

53. T.J. Batterham, private communication.

54. A.I. Scott, "Interpretation of Ultraviolet Spectra of Natural Products", Pergamon Press, 1964, p.290.

55. J.H. Beynon and A.E. Williams, J. Applied Spectroscopy, 14, 156 (1960).

56. K. Venkataraman, "The Chemistry of Flavonoid Compounds", ed. T.A. Geissman, Pergamon Press, 1962, p.70.

57. M. Shimokoriyama, J. Amer. Chem. Soc., 79, 4199 (1957).

58. G.B. Marini-Bettolo and A. Ballio, Gazz. chim. ital., 76, 410 (1946).

59. K.R. Markham and T.J. Mabry, Phytochem., 7, 791 (1968).

60. L. Jurd, Arch. Biochem. Biophys., 63, 376(1956).

61. N.B. Colthup, L.H. Daly and S.E. Wiberley, "Introduction to Infrared and Raman Spectroscopy", Academic Press, 1964, p.226.

62. J. Seibl, Helv. Chim. Acta., 50, 264 (1967).

63. K.R. Jennings, Chem. Comm., 283 (1966).

64. S. O'Brien and D.C.C. Smith, J. Chem. Soc., 2907 (1963).

65. I.F.B. Common, Division of Entomology, C.S.I.R.O., Canberra, private communication.

66. W.T.K. Hall, Queensland J. Agr. Sci., 13, 97 (1956). [Chem.Abs., 51, 1503 (1957)]

67. H. Budzikiewicz, C. Djerassi and D.H. Williams, "Interpretation of Mass Spectra of Organic Compounds", Holden-Day, San Francisco, 1964, p.169.

68. I. Heilbron, A.H. Cook, H.M. Bunbury and D.H. Hey, "Dictionary of Organic Compounds", Eyre and Spottiswoode, London, 4th edn., 1964.

69. M. Uchibayashi and T. Matsuoka, Chem. and Pharm. Bull. (Tokyo), 9, 234 (1961).

70. O. Hesse, Annalen, 309, 32 (1899).

71. P.A.A.F. Eijken, Pharm. Weekblad, 41, 177, 197 (1904).

72. A. Tschirch and P.A.A.F. Eijken, Schweiz. Wochshr. Pharm., Nos. 40 and 41 (1904).

73. F. Tutin and H.W.B. Clewer, J. Chem. Soc., 99, 946 (1911).

74. (a) O. Hesse, J. prakt. Chem., 77, 321 (1908). [Chem.Abs., 2, 2282 (1908)]

(b) Idem, ibid., 77, 383 (1908). [Chem.Abs., 2, 2556 (1908)]

75. (a) S. Keimatsu and I. Hirano, J. Pharm. Soc. Japan, 49, 147 (1929). [Chem.Abs., 23, 3464 (1929)]

(b) Idem, ibid., 51, 230 (1931). [Chem.Abs., 25, 3647 (1931)]

76. C. Schuerch, J. Amer. Chem. Soc., 72, 3838 (1950).

77. H.M. Hurst and J.B. Harborne, Phytochem., 6, 1111 (1967).

78. N. Mauthner, Chem.Abs., 42, 1240 (1948).

79. J. Chopin and M.L. Bouillant, Compt.rend., 252, 2727 (1961).

80. (a) B.D. Davis, Advances in Enzymol., 16, 247 (1955).

(b) Idem, Arch. Biochem. Biophys., 78, 497 (1958), and references therein.

81. H. Plieninger, Angew. Chem., 74, 423 (1962).

82. (a) J.D. Bu'Lock, "The Biosynthesis of Natural Products", McGraw-Hill, 1965, p.78.

(b) J.B. Harborne, "Biochemistry of Phenolic Compounds",Academic Press London, 1964.

(c) T. Swain, "Chemical Plant Taxonomy", Academic Press, London, 1963.

(d) T.A. Geissman, "The Chemistry of Flavonoid Compounds", Pergamon Press, 1962.

83. J.N. Collie, J. Chem. Soc., 1806 (1907).

84. A.J. Birch and F.W. Donovan, Aust. J. Chem., 6, 360 (1953).

85. G. Linstedt and A. Misiorny, Acta Chem. Scand., 5, 121 (1951).

86. H. Erdtman, "Perspectives in Organic Chemistry", Interscience, New York, 1956, p.453.

87. A.J. Birch, Annual Review of Plant Physiology, 19, 321 (1968).

88. (a) A.J. Birch, Fortschr. Chem. Org. Naturstoffe, 14, 186 (1957).

(b) A.J. Birch and H. Smith, Chem. Soc. Special Publ., 12, 1 (1958).

(c) A.J. Birch, Proc. Chem. Soc. Lond., 3 (1962).

(d) Idem, Science, 156, 233 (1967).

89. R.W. Rickards, "Recent Developments in the Chemistry of Natural Phenolic Products", ed. W.D. Ollis, Pergamon Press, 1961.

90. A.J. Birch, R.A. Massy-Westropp, R.W. Rickards and H. Smith, J. Chem. Soc., 360 (1958).

91. A.J. Birch, A.J. Ryan and H. Smith, J. Chem. Soc., 4773 (1958).

92. A.J. Birch, P. Fitton, E. Pride, A.J. Ryan, H. Smith and W.B. Whalley, J. Chem. Soc., 4576 (1958).

93. (a) A.J. Birch, G.E. Blance and H. Smith, J. Chem. Soc., 4582 (1958).

(b) K. Mosbach, Acta Chem. Scand., 14, 457 (1960).

94. F.M. Dean, ref. 41, p.335.

95. E. Moustafa and E. Wong, Phytochem., 6, 625 (1967).

96. H. Meier and H.M. Zenk, Z. Pflanzenphysiol., 53, 415 (1965).

97. (a) E.W. Underhill, J.E. Watkin and A.C. Neish, Canad. J. Biochem. and Physiol., 35, 219 (1957).

(b) Idem, ibid., 35, 229 (1957).

98. A.J. Birch, "Proc.Intern.Congr.Pure Appl.Chem., 17th Plenary Lectures", Butterworths, London, 1961, p.73.

99. L. Patschke, W. Barz and H. Grisebach, Z. Naturforsch., 21b, 201 (1966).

100. L. Patschke and H. Grisebach, Z. Naturforsch., 20b, 399 (1965).

101. H. Grisebach and S. Kellner, Z. Naturforsch., 20b, 446 (1965).

102. L. Patschke, W. Barz and H. Grisebach, Z. Naturforsch., 21b, 45 (1966).

103. W. Barz and H. Grisebach, Z. Naturforsch., 21b, 47 (1966).

104. A. Pelter, Tetrahedron Letters, 897 (1968).

105. M. Sribney and S. Kirkwood, Nature, 171, 931 (1953).

106. D.H.R. Barton, G.W. Kirby and J.B. Taylor, Proc. Chem. Soc. Lond., 340 (1962).

107. W. Baker, W.D. Ollis and K.W. Robinson, Proc. Chem. Soc. Lond., 269 (1959).

108. Y. Fukui and N. Kawano, J. Amer. Chem. Soc., 81, 6331 (1959).

109. (a) B. Jackson, H.D. Locksley and F. Scheinmann, Tetrahedron Letters, 787 (1967).

(b) A. Pelter, Tetrahedron Letters, 1767 (1967).

(c) B. Jackson, H.D. Locksley, F. Scheinmann and W.A. Wolstenholme, Tetrahedron Letters, 3049, (1967).

(d) H. Grisebach, Tetrahedron Letters, 4095 (1967).

(e) B. Jackson, H.D. Locksley and F. Scheinmann, Chem.Comm., 1125 (1968).

110. H. Miura, T. Kihara and N. Kawano, Tetrahedron Letters, 2339 (1968).

111. N. Kawano and M. Yamada, J. Amer. Chem. Soc., 82, 1505 (1960).

112. V.V.S. Murti, P.V. Raman and T.R. Seshadri, Tetrahedron, 23, 397 (1967).

113. C.G. Karanjgaokar, P.V. Radhakrishnan and K. Venkataraman, Tetrahedron Letters, 3195 (1967).

114. A. Russell and J. Todd, J. Chem. Soc., 421 (1937).

115. T.A. Geissman and H.F.K. Dittmar, Phytochem., 4, 359 (1965).

116. L. Jurd, Tetrahedron, 23, 1057 (1967).

117. S. Rangaswami and P. Venkateswarlu, Chem.Abs., 66, 65364 (1967).

118. R. Hansel and G. Schopflin, Tetrahedron Letters, 37, 3645 (1967).

119. K. Freudenberg and K. Weinges, Chem.Abs., 66, 55333 (1967).

120. S.E. Drewes, D.G. Roux, J. Feeney and S.H. Eggers, Chem. Comm., 368 (1966).

121. E. Wenkert, Chem. and Ind., 906 (1959).

122. A. Pelter and R. Hansel, Tetrahedron Letters, 2911 (1968).

123. R.B. Woodward, Angew. Chem., 69, 50 (1957).

124. W.B. Whalley, "Recent Developments in the Chemistry of Natural Phenolic Compounds", ed. W.D. Ollis, Pergamon Press, 1961, p.55.

125. E.C. Bate-Smith, "Chemical Plant Taxonomy", ed. T. Swain, Academic Press, 1963, p.130.

126. A.T. Lee, Contributions from the N.S.W. National Herbarium, 4, 35 (1966).

127. Idem, ibid., Flora Series, No. 34 (1966).

128. E.C. Bate-Smith, ref.125, p.138.

129. St.v. Kostanecki and J. Tambor, Ber., 2260 (1899).

130. M. Hasegawa and T. Shirato, J. Amer. Chem. Soc., 79, 450 (1957).

131. K.C. Gulati, S.R. Seth and K. Venkataraman, Org. Synth., Coll. Vol. 2, p.522.

132. E.P. Kohler and H.M. Chadwell, Org. Synth., Coll. Vol. 1, 2nd edn., p.78.

133. C. Enebäck and J. Gripenberg, Acta Chem. Scand., 11, 866 (1957).

134. W.H. Hartung, Org. Synth., 26, 32 (1946).

135. J. Massicot, C. Mentzer and D. Pillon, Compt. rend., 238, 111 (1954).

136. R.N. Goel and T.R. Seshadri, Tetrahedron, 5, 91 (1959).

137. S. Asen and L. Jurd, Phytochem., 6, 577 (1967).

138. A.I. Vogel, Practical Organic Chemistry, Longmans, 3rd edn., p.971.

139. L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Wiley and Sons, 1967, p.295.

140. K. Randerath, Thin-layer Chromatography, Academic Press, (1966).

141. E. Stahl, Thin-layer Chromatography, Academic Press (1965).

142. H.R. Arthur, W.H. Hui and C.N. Ma, J. Chem. Soc., 632 (1956).